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Genomic clone and promoter capable of tissue- and development-specific expression - derived from e.g. Brassica campestris, useful in prodn. of male sterile transgenic plants
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EP 674711	A1	19951004	WO 93AU657	A	19931216	C12N-015/29	199544
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Abstract (Basic): WO 9413809 A
A genomic DNA isolate is claimed comprising (i) all or part of a gene preferentially expressed in anther tissue of a plant and not in non-anther tissue; (ii) an open reading frame (ORF) having the nucleotide sequence given in the specification or having at least 20% similarity; and opt. (iii) a promoter region 5' to the ORF.
USE/ADVANTAGE - The identification of a tissue and developmentally dependent promoter enables the production of genetic constructs which can be used to generate transgenic plants having certain traits expressed or down regulated. For example, the function of a gene can be conveniently disrupted using antisense RNA or a ribozyme. The cDNA clone Bcp1 inserted in the reverse orientation relative to the Bgp1 promoter, when introduced into a suitable host, produces antisense RNA which disrupts expression of the Bgp1 gene. It is possible the antisense RNA forms a duplex with Bgp1 RNA preventing its translation. Transgenic plants carrying the particular construct are generally male sterile but female fertile.
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Title Terms: GENOME; CLONE; PROMOTE; CAPABLE; TISSUE; DEVELOP; SPECIFIC; EXPRESS; DERIVATIVE; BRASSICA; CAMPESTRIS; USEFUL; PRODUCE; MALE; STERILE; TRANSGENIC; PLANT
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(54) Title: DEVELOPMENTAL REGULATION IN ANTHER TISSUE OF PLANTS

(57) Abstract

The present invention relates generally to the use of genetic engineering to induce developmental regulation in anther tissue of plants, and more particularly to induce nuclear male sterility, and to genetic sequences useful for same. More particularly, the present invention relates to the identification of a genomic clone and promoter capable of tissue- and development-specific expression which provides a means of tissue and developmental regulation in plants and more specifically a means of producing nuclear male sterile plants. Even more particularly, the present invention provides a genomic clone having a nucleotide sequence as set forth in SEQ ID NO.1 or homologous sequences thereof such as the nucleotide sequence as set forth in SEQ ID NO.3.

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DEVELOPMENTAL REGULATION IN ANTHER TISSUE OF PLANTS

The present invention relates generally to the use of genetic engineering to induce developmental regulation in anther tissue of plants, and more particularly to induce nuclear male sterility, and to genetic sequences useful for same.

Nucleotide and amino acid sequences are referred to herein by sequence identity numbers (SEQ ID NOs) which are defined after the bibliography. A general summary of the SEQ ID NOs is provided before the examples.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Anther-specific genes are those genes that are expressed exclusively in the male reproductive tissues, rather than "house-keeping" genes which are active in all plant cells. Anther-specific genes play an important role in pollen development and, hence, in the control of seed production.

Differentiation and development of the male gametophyte of angiosperms, the pollen grain, depends partly upon transcription of the haploid genome following meiosis (Mascarenhas, 1988). The study of these coordinated events at the molecular level has been considered important in order to understand the developmentally specific regulation and functions of pollen-expressed genes. In this regard, Theerakulpisut *et al* (1991) studied gene expression in pollen of Brassica campestris. By differential screening of a mature B. campestris pollen cDNA library, an anther-specific clone, designated Bcp1, was isolated.

In work leading up to the present invention, the inventors undertook a detailed

investigation of Bcp1 expression with the aim of isolating a genomic clone and to sub-clone and characterise the 5′ upstream regulatory regions of the genomic clone. It has been surprisingly discovered that the genomic clone of Bcp1, i.e. Bgp1, is tissue and developmentally specific thereby providing a means to enable tissue and developmental regulation in plants and in particular to produce nuclear male sterile plants. It has further been discovered that the Bgp1 gene from *B. campestris* represents a family of homologous genes from a diverse range of plants. By way of shorthand notation, a genomic clone is referred to herein by the genus and/or species of the plant from which it is isolated followed by the term "Bgp1". A cDNA clone is referred to in similar fashion except using the term "Bcp1".

Accordingly, one aspect of the present invention contemplates a genomic DNA isolate comprising:

(i) all or part of a gene or related genetic sequence preferentially expressed in anther tissue of a plant and substantially not expressed in non-anther tissue; and

(ii) an open reading frame having a nucleotide sequence as set forth in SEQ ID NO. 1:

```
ATG GGT CGC CAA AAC GCT GTC GTA GTT TTT GGC CTT GTG TTC TTG GCC
ATC CTT GGC CTC GCC GCA GCT GCC TCC TCT CCG TCT CCT TCA GCG TCA
CCC TCC AAA GCT CCG GCT GCT ACC GTA ACC GAT GTC GAA GCT CCA GTG
AGC GAG GAC ACC ATT GGA ACC ACC GAT GAC GAT GCA GCT GCT TCT CCA
GGT GAT GGT GAC GTA GCT GTG GCT GGT CCT CTA GGA AGT GAC TCC TCC
TAC GGT AGT AAT GGA CCT TCA CCT TCT ACT GAT GCT GCT GAC AGC GGC
GCG CCT GCT CTT GGC GTC TCT GCG GTC TTC GTT GGT GTT GCA TCC ATC
GCC GGT TCT TTC TTG TTT CTC
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or having at least 20% similarity to all or part thereof.

The deduced amino acid sequence to the open reading frame defined in SEQ ID NO. 1 is shown in SEQ ID NO. 2.

The expression "gene or related genetic sequence" is used in is broadest sense and includes any contiguous series of nucleotides constituting an open reading frame.

Generally, an open reading frame comprises at least 48 contiguous nucleotides arranged into triplets without interuption by a stop codon.

A nucleotide sequence having at least 20% similarity to all or a portion of SEQ ID NO. 1 is referred to herein as a "homologous gene". Preferably, there is at least 20% similarity to the entire SEQ ID NO. 1 sequence. Even more preferably, there is at least 30% similarity, still more preferably at least 45% similarity, even still more preferably at least 55-60% similarity, yet even still more preferably at least 75-95% similarity to all or part of SEQ ID NO. 1. A "part" in this context is a contiguous series of at least 20 nucleotides in SEQ ID NO. 1.

Preferably, the genomic DNA isolate is a dicotyledonous plant such as tomato, corn, rice, wheat, raddish, tobacco and oil seed rapes. Particularly preferred plants are *Brassica* species, *Arabidopsis* species and *Nicotiana* species.

In a most preferred embodiment, the plant is ***Brassica campestris*** and the genomic DNA isolate has an open reading frame with a sequence as set forth in SEQ ID NO. 1. A preferred homologous gene having at least 20% nucleotide similarity to SEQ ID NO. 1 is from ***Arabidopsis thaliana*** comprising an open reading frame with a nucleotide sequence as set forth in SEQ ID NO. 3:

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ATG GGT CGC CAA AAC ATT GTC GTC GTG GTT GCC CTC GTC TTC ATC CGG
ATC ATT GGC CTT GCC GCA GCT GCC TCC TCT CCA TCT CCT TCA GCG TCT
CCC TCC AAA GCT CCA GCT GCC TCC AAA ACC GAT CAT GTC GAG GCT CCA
GTC ACC GAT GAC CAA ATC GGA ACC ACC GAT GAC GAT GCA GCT CCT ACT
CCT GGT GAC GGT GAC GTT GCA GTG GCT GGT CCT CTA GGA AGT GAC TCC
TCG TAC GAC AAT GCC GCT ACA GGC TCT GCT GAT TCT GCC AAA AGC GGT
GCG GCA GCT CTT GGC GTC TCT GCG GTC GTC GTT GGT GTT ACA TCA TTG
CTG GTT CTT TCT TGT TAC TCA AGT TGG GCA TTG TTT TAT GAT AAG AAG
GTT ATT TTA AAC GAA GAT TAT TAT ATG
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The deduced amino acid sequence of SEQ ID NO. 3 is defined in SEQ ID NO. 4.

Another aspect of the present invention provides a genomic DNA isolate as defined above and further comprising a promoter region 5′ to the open reading frame,

wherein said promoter region:

(i) is capable of directing expression in taptum and/or pollen tissue; and

(ii) comprises a nucleotide sequence as set forth in SEQ ID NO. 5:

TATCATTCCT TTAATTTCAA GGAATTATAG AACAAAAAAT GTTCTTTATA AAAATTAAGA

AGGAACAAGG GATTCATTCC TACTATTCTG TTCTTGGTCA TTATTTTCCT CTTCATTCAT

ATTGTTTCTT TAATTGTTAC CAATTAGAAC TTTAACGAAT AAATAGTTAA TTCGTATTAT

GAGATTTACA CAATTCTTAT TCACTCAATT TGGAGTTTTA AAGATTTTTT AAAAGATTTA

TGGTGGGAAC CTTCTTCTTT TCTTATTTAT CATGATGATG ATAACCTTCC CAGCAGAATT

ATTCTTAGAA CTTTTTTTCA CATTTAGGTA TCCATGCCTA AGTAAGGCTT AGTTAAAGAT

GTTTTATAAA CTTTGATCAA AATATTCATT CAATTAATTT GAGCTTCAAC TATAAATTGT

TGTATGCATT CGTTTTAGCC TGTAAGATAT CAGACATTCA CGTTTCGATA AACAAGTATA

TAAATAATAT GAATATTGTA CATTCATTTT ATTCGGTTCA TCAACCAAAA AAAATAAAAA

TAAATATTCG TATTCATCTA TGCTTTGGCA TGGTCCGTTC TTTTTTCTTG ATTGGCTCGT

TACCATTCAA AAATATATAC CTTAGCAAAC CCATTTTTAG ACATTCCAGT TGATCTACAT

TAGATTGAAC GGTATTCCTC CTACGTAGTA AGAACGTTTT CTATTTTTCT TTGTTTCAGT

CATACAACAC AACTATATAT ACACAGCAAC CCCATCTCCT CTCCAATCAT CACAATCTCT

AACGTTAAAC CCTAAGACAA ACTAAAAGAG AGCTACGTAC AAGGAGACAG AGAGAAGA

or having at least 20% similarity to all or part thereof.

Preferred promoters comprise the promoter defined in SEQ ID NO. 5 and the promoter defined in SEQ ID NO. 6 which has the following nucleotide sequence:

AAAAGCGAGA AGAAGAAGTC TGGAAGATTT GAGAGCTTAA AGTGGTCGAG TGTAAAACCC

TAACTCGCTG TTGATGGCAG AATCGTAAAT CGGAATTGAT TCATGGGCCT AACAAGACGT

TTGGGCTTAT GGGTTTAAAG CCCATCTGAT ATAAGATGAA TAGAATGTTC ATGGCAATAC

TATCATAATT TGGTTCTTTA ATAAGACACT CGTTAATACG ACGACGATTT GAAGTTGAAC

GAATGTTTTC ATATTCATTC GCATGTTCAC CAATCAAAAT CTATATCTCA ACAAGTCCAT

TTTTAGGTAC TCCAGTAGAT TTACATTGGA TTGTAAGGTA ATCCTACATC TTAGTTCACG

TTTTCTATTT TTGGTCTTGT CACTAAACAC AACTATATAT ACATATCAAA CTCATCTTCG

GAAATCATCA CAATCAATAA ACCTCAAACC CTAAAATAAA TTAAACGAGT TCTACGTAAG

AAGGAGAGAG AGAAGA

Yet another aspect of the present invention relates to a genomic DNA isolate comprising:

(i) all or part of a gene or related genetic sequence preferentially expressed in anther tissue of a plant and substantially not expressed in non-anther tissue;

(ii) a promoter region capable of directing expression in tapetum and/or pollen tissue;

(iii) a nucleotide sequence substantially as set forth in SEQ ID NO. 7 :

TATCATTCCT TTAATTTCAA GGAATTATAG AACAAAAAAT GTTCTTTATA AAAATTAAGA

AGGAACAAGG GATTCATTCC TACTATTCTG TTCTTGGTCA TTATTTTCCT CTTCATTCAT

ATTGTTTCTT TAATTGTTAC CAATTAGAAC TTTAACGAAT AAATAGTTAA TTCGTATTAT

GAGATTTACA CAATTCTTAT TCACTCAATT TGGAGTTTTA AAGATTTTTT AAAAGATTTA

TGGTGGGAAC CTTCTTCTTT TCTTATTTAT CATGATGATG ATAACCTTCC CAGCAGAATT

ATTCTTAGAA CTTTTTTTCA CATTTAGGTA TCCATGCCTA AGTAAGGCTT AGTTAAAGAT

GTTTTATAAA CTTTGATCAA AATATTCATT CAATTAATTT GAGCTTCAAC TATAAATTGT

TGTATGCATT CGTTTTAGCC TGTAAGATAT CAGACATTCA CGTTTCGATA AACAAGTATA

TAAATAATAT GAATATTGTA CATTCATTTT ATTCGGTTCA TCAACCAAAA AAAATAAAAA

TAAATATTCG TATTCATCTA TGCTTTGGCA TGGTCCGTTC TTTTTTCTTG ATTGGCTCGT

TACCATTCAA AAATATATAC CTTAGCAAAC CCATTTTTAG ACATTCCAGT TGATCTACAT

TAGATTGAAC GGTATTCCTC CTACGTAGTA AGAACGTTTT CTATTTTTCT TTGTTTCAGT

CATACAACAC AACTATATAT ACACAGCAAC CCCATCTCCT CTCCAATCAT CACAATCTCT

AACGTTAAAC CCTAAGACAA ACTAAAAGAG AGCTACGTAC AAGGAGACAG AGAGAAGAAT

GGGTCGCCAA AACGCTGTCG TAGTTTTTGG CCTTGTGTTC TTGGCCATCC TTGGCCTCGC

CGCAGCTGCC TCCTCTCCGT CTCCTTCAGC GTCACCCTCC AAAGCTCCGG CTGCTACCGT

AACCGATGTC GAAGCTCCAG TGAGCGAGGA CACCATTGGA ACCACCGATG ACGATGCAGC

TGCTTCTCCA GGTGATGGTG ACGTAGCTGT GGCTGGTCCT CTAGGAAGTG ACTCCTCCTA

CGGTAGTAAT GGACCTTCAC CTTCTACTGA TGCTGCTGAC AGCGGCGCGC CTGCTCTTGG

CGTCTCTGCG GTCTTCGTTG GTGTTGCATC CATCGCCGGT TCTTTCTTGT TTCTCTGAGG

TGTGTATTAT CATGAGAAGA TTATTCTGAC TGAAGACTAT TAATATGTAT GGATGATTGT

GATGGTCGTG TTGTAATATG TTTCTCCTTT ATTGTGAGAA ACGATGTTTT GCTAATAAAA

CTGAAAAAAA AAACGAAAAT TTCCTCTAGC CAAGGATAAA ATGCCGGAAT TGCGGATTAA

ATAGTACTAT TCAATCCTTT CATGTTTTCG AGATACAAAA ATACATATTA ATCAGGTAGA

GCCGTAGAAG TCCGTAACCA CTGGATACAA TCTTTTTCGT AGTAAGAAAG AAAGTACAAT

CTTATTCTAA ATGCATGTGT TTGATAGATT ATGGAACGGT GAGAAGGGCA TTGATTATGG

GAGTTATGAT CGAAGATACA CACGATACCA TCTTTTTAGG TATAGCTTCT TCTTCTATAA

A

or having at least 20% similarity to all or part thereof.

In a preferred embodiment, the above genomic DNA isolate further comprises:

(iv) a nucleotide sequence which is capable of hybridising under low stringency conditions to all or part of a nucleotide sequence substantially complementary to SEQ ID NO. 7.

Most preferred genomic DNA isolates comprise SEQ ID NO. 7 and SEQ ID NO. 8, the latter which has the following nucleotide sequence:

AAAAGCGAGA AGAAGAAGTC TGGAAGATTT GAGAGCTTAA AGTGGTCGAG TGTAAAACCC

TAACTCGCTG TTGATGGCAG AATCGTAAAT CGGAATTGAT TCATGGGCCT AACAAGACGT

TTGGGCTTAT GGGTTTAAAG CCCATCTGAT ATAAGATGAA TAGAATGTTC ATGGCAATAC

TATCATAATT TGGTTCTTTA ATAAGACACT CGTTAATACG ACGACGATTT GAAGTTGAAC

GAATGTTTTC ATATTCATTC GCATGTTCAC CAATCAAAAT CTATATCTGA ACAAGTCCAT

TTTTAGGTAC TCCAGTAGAT TTACATTGGA TTGTAAGGTA ATCCTACATC TTAGTTCACG

TTTTCTATTT TTGGTCTTGT CACTAAACAC AACTATATAT ACATATCAAA CTCATCTTCG

GAAATCATCA CAATCAATAA ACCTCAAACC CTAAAATAAA TTAAACGAGT TCTACGTAAG

AAGGAGAGAG AGAAGAATGG GTCGCCAAAA CATTGTCGTC GTGGTTGCCC TCGTCTTCAT

CCGGATCATT GGCCTTGCCG CAGCTGCCTC CTCTCCATCT CCTTCAGCGT CTCCCTCCAA

AGCTCCAGCT GCCTCCAAAA CCGATCATGT CGAGGCTCCA GTCACCGATG ACCAAATCGG

AACCACCGAT GACGATGCAG CTCCTACTCC TGGTGACGGT GACGTTGCAG TGGCTGGTCC

TCTAGGAAGT GACTCCTCGT ACGACAATGC CGCTACAGGC TCTGCTGATT CTGCCAAAAG

CGGTGCGGCA GCTCTTGGCG TCTCTGCGGT CGTCGTTGGT GTTACATCAT TGCTGGTTCT

TTCTTGTTAC TCAAGTTGGG CATTGTTTTA TGATAAGAAG GTTATTTTAA ACGAAGATTA

TTATATGTAA GGATGATTGT GATGATCCGT TGACCTGCAG GTCGACCCAG ATCCGCCTAC

CTTTCACGAG TTGCGCAGTT TGTCTGCAAG ACTCTATGAG AAGCTGATAA GAGATAAGTT

TGCTCAACAT CTTCTCGGGC ATAAGTCCGG ACACCATGGC ATCACAGTAT CGAGATGACA

GAGGCAGGGA GTGGGACAAA ATTGAAATCA AATGATCGAT TTTATTTTGG CT

Still yet another aspect of the present invention contemplates an isolated nucleic acid molecule which is capable of hybridising under low stringency conditions to the genomic DNA isolates defined above. Preferred nucleic acid molecules comprise a complementary strand of all or part of SEQ ID NO. 1 or SEQ ID NO. 3. A "part" in this context includes an oligonucleotide.

A further aspect of the present invention provides a genetic construct comprising:

(i) a promoter region capable of directing expression of a nucleotide sequence when operably linked downstream thereof in tapetum and/or pollen tissue; and

(ii) said promoter being capable of hybridising under low stringency conditions to a complementary strand of SEQ ID NO. 5.

For the purposes of defining the level of stringency, reference can conveniently be made to Sambrook *et al.*, *Supra* at pp 387-389 which is herein incorporated by reference where the washing step at paragraph 11 is considered high stringency. A low stringency is defined herein as being in 0.1-0.5% w/v SDS at 37-45 °C for 2-3 hours. Depending on the source and concentration of nucleic acid involved in the hybridisation, alternative conditions of stringency may be employed such as medium stringent conditions which are considered herein to be 0.25%-0.5% w/v SDS at ≥ 45 °C for 2-3 hours or high stringent conditions as disclosed by Sambrook *et al.*, *Supra*.

In a further related embodiment, there is provided a nucleic acid isolate having a sequence of nucleotides comprising or a complementary sequence of nucleotides comprising SEQ ID NO. 5 or a promoter functional derivative, fragment, part, homologue or analogue thereof. The latter functional derivative and like molecules comprise at least 20% nucleotide sequence similarity to SEQ ID NO. 5. An example of a promoter having at least 20% nucleotide similarity to SEQ ID NO. 5 is the promoter from *A. thaliana* Bgp1 having the sequence set forth in SEQ ID NO. 6.

In accordance with these and other aspects of the present invention, the term "promoter" is used in its most general sense and refers to any nucleotide sequence which binds RNA polymerase and directs same to a transcriptional start site whereupon a gene or other nucleotide sequence downstream of said promoter is transcribed. A nucleotide sequence "downstream" of the promoter is also said to be "relative" the promoter.

The term "genetic construct" is used in its most broadest sense to include an isolated nucleic acid molecule comprising a sequence of nucleotides.

Preferably, the promoter is from a *Brassica* species such as *B. compestris* or from an *Arabidopsis* species such as *A. thaliana*. Preferably, the genetic construct is transformable and operable in dicotyledon plants and in particular a *Brassica* species, *Arabidopsis* species or a *Nicotiana* species.

The genetic construct may be conveniently engineered so as to place an endonuclease restriction site in a region 3′ of the promoter to thereby readily enable the insertion of nucleotide sequences downstream of the promoter for their transcription. Generally, the inserted restriction site is unique to the genetic construct or may be represented twice but separated by a length of nucleic acid to be deleted upon restriction digestion of the genetic construct and followed by insertion of the required nucleotide sequence to be transcribed.

The genetic construct of the present invention may comprise solely the promoter and optionally a nucleotide sequence downstream thereof or, alternatively, may comprise additional nucleotide sequences constituting promoter regulatory region(s), transcribed sequence regulatory regions, a marker (eg. antibiotic resistance, chemical compound resistance or enzyme), autonomous replication region and/or genome integration sequence. The promoter may be the naturally occurring promoter or may be an active fragment or part thereof or a derivative, analogue or homologue of the promoter.

By "derivative" is meant to include any single or multiple nucleotide deletion, insertion and/or substitution to the promoter nucleotide sequence, provided said derivative is still active in tapetum and/or pollen tissue. Manipulation of the nucleotide sequence at known predetermined sites or random mutagenesis are conveniently accomplished by any number of techniques including M13, transposon and/or oligonucleotide mutagenesis. Various techniques are described by Maniatis *et al* (1989).

Homologues and analogues of the promoter include promoters having a nucleotide sequence having at least 20%, preferably at least 30% similarity, more preferably at least 45% similarity, still more preferably at least 55-60% similarity and even more preferably at least 75-95% similarity to the first mentioned promoter and which function in anther tissue.

Most preferred promoters comprise the sequence SEQ ID NO. 5 or SEQ ID NO. 6.

The promoter of the present invention is tissue specific for anther tissue. More particularly, the promoter is specific for tapetum and/or pollen tissue. However, this is not intended to exclude genetic constructs based on the promoter of the present invention but modified to be capable of expression in non-anther tissues.

The nucleotide sequence down stream of the promoter might give rise to antisense RNA or may encode specific traits such as a "lethal gene" or a "killer gene" to specifically render a pollen grain infertile or incapable of maturation. The nucleotide sequence may also encode a trait, for example, which renders the pollen grain more resistant to predator or pathogen attack. In one particular embodiment, the nucleotide sequence downstream of the promoter is a ribozyme capable of targetting a mRNA transcript corresponding to SEQ ID NO. 1 or SEQ ID NO. 3 or a homologous genetic sequence thereof.

According to this latter embodiment, there is provided a ribozyme which comprises a hybridising region and a catalytic region wherein the hybridising region is capable of hybridising to at least part of a target mRNA sequence transcribed from a genomic Bgp1 gene as hereinbefore defined wherein the catalytic region is capable of cleaving said target mRNA thereby substantially down regulating expression of said genomic DNA isolate. A ribozyme according to this aspect of the invention may also be a polyribozyme.

Methods for the construction of ribozyme are conveniently disclosed in Haseloff and Gerlach (1988) and in International Patent Application No. WO89/05852. Preferably, the ribozyme is under the control of a Bgp1 promoter as hereinbefore described.

The present invention further extends to a hybrid genetic sequence comprising a ribozyme as hereinbefore described fused, linked or otherwise chemically bonded to one or more sequence of nucleotides which is/are substantially antisense to all or part of SEQ ID NO. 1 or a homologous sequence (e.g. antisense to all or part of SEQ ID NO. 3).

The antisense sequence may flank both sides of a ribozyme or may be located to one end of said ribozyme. Reference to a ribozyme in this context includes reference to a polyribozyme. A "substantially antisense" molecule is a molecule capable of hybridising under physiological conditions to the reference sequence (e.g. SEQ ID NO. 1 or SEQ ID NO. 3) to a sufficient extent to reduce translation of said target sequence into functional protein or which results in male sterility.

The present invention is particularly exemplified using the promoter isolated from a genomic clone of Bcp1, the genomic clone being designated herein "Bgp1", from Brassica species or non-Brassica species with similar acting promoters. Such other promoters are referred to herein as "homologous promoters" and include the promoter from the homologous gene *A. thaliana* Bgp1 defined by SEQ ID NO. 6.

Most of the Bgp1 promoter is required for pollen expression and in particular nucleotide regions -580 to -767, -322 to -580 and -116 to -168 whereas the nucleotide region up to -116 is only required for tapetum expression.

According to a preferred embodiment, the present invention provides an isolated nucleic acid molecule carrying a promoter capable of directing expression in tapetum and pollen tissue and comprising the following nucleotide sequence identified as SEQ ID NO. 5, including functional derivatives or homologues having at least 20% nucleotide similarity to all or a part thereof and/or which are capable of hybridising to a complementary strand thereof under at least low stringency conditions.

According to another embodiment, there is provided an isolated nucleic acid molecule carrying a promoter capable of directing expression in pollen tissue but not tapetum, said nucleic acid molecule comprising the following nucleotide sequence identified as SEQ ID NO. 9:

TATCATTCCT TTAATTTCAA GGAATTATAG AACAAAAAAT GTTCTTTATA AAAATTAAGA

AGGAACAAGG GATTCATTCC TACTATTCTG TTCTTGGTCA TTATTTTCCT CTTCATTCAT

ATTGTTTCTT TAATTGTTAC CAATTAGAAC TTTAACGAAT AAATAGTTAA TTCGTATTAT

GAGATTTACA CAATTCTTAT TCACTCAATT TGGAGTTTTA AAGATTTTTT AAAAGATTTA

TGGTGGGAAC CTTCTTCTTT TCTTATTTAT CATGATGATG ATAACCTTCC CAGCAGAATT

ATTCTTAGAA CTTTTTTTCA CATTTAGGTA TCCATGCCTA AGTAAGGCTT AGTTAAAGAT

GTTTTATAAA CTTTGATCAA AATATTCATT CAATTAATTT GAGCTTCAAC TATAAATTGT

TGTATGCATT CGTTTTAGCC TGTAAGATAT CAGACATTCA CGTTTCGATA AACAAGTATA

TAAATAATAT GAATATTGTA CATTCATTTT ATTCGGTTCA TCAACCAAAA AAAATAAAAA

TAAATATTCG TATTCATCTA TGCTTTGGCA TGGTCCGTTC TTTTTTCTTG ATTGGCTCGT

TACCATTCAA AAATATATAC CTTAGCAAAC CCATTTTTAG ACATTCCAGT TG

including functional derivatives or homologues having at least 20% nucleotide similarity to all or a part thereof and/or which are capable of hybridising to a complementary strand thereof under at least low stringency conditions.

A particularly important homologue is SEQ ID NO. 6 from *A. thaliana*.

Preferably, the nucleotide sequence of SEQ ID NO. 5, SEQ ID NO. 9 or SEQ ID NO. 6 is modified by the introduction of a restriction endonuclease cleavage site to facilitate the insertion of an operably linked second nucleotide sequence downstream of the promoter.

Preferably, the nucleotide sequence of the present invention form part of a vector.

The identification of a tissue and developmentally dependent promoter enables the production of genetic constructs which can be used to generate transgenic plants having certain traits expressed or down regulated. For example, the function of the Bgp1 gene can be conveniently disrupted using antisense RNA or a ribozyme. Conveniently, the cDNA clone Bcp1 is inserted in the reverse orientation relative the Bgp1 promoter. This construct, when introduced into a suitable host, produces antisense RNA which disrupts expression of the Bgp1 gene. Although not intending to limit the present invention to any one theory of mode of action, it is possible the antisense RNA forms a duplex with Bgp1 RNA to thereby prevent its translation. Transgenic plants carrying the particular construct are generally male sterile but female fertile.

According to this aspect of the present invention there is provided an antisense construct:

- (i) which comprises a nucleic acid molecule comprising at least eight contiguous nucleotides;
- (ii) which is capable of hybridising under physiological conditions to all or part of SEQ ID NO. 1 or a homologous sequence thereof; and
- (iii) which, in use, is capable of down regulating expression of a plant Bgp1

gene.

In this context, a "homologous" sequence comprises a nucleotide sequence having at least 20% similarity to all or part of SEQ ID NO. 1 and which is a Bgp1 gene.

Preferably, the antisense construct is at least 20 nucleotides long. More preferably, the antisense construct is at least 50-100 nucleotides long. Even more preferably, the antisense construct is all or part of a plant Bcp1 or Bgp1 in reverse orientation relative a promoter.

The term "down regulates" or similar expressions such as "down regulating" means a reduction in the amount of full length Bgp1 mRNA as determined by hybridisation or extent of translation into a Bgp1 product or, most conveniently, generation of substantially male sterile plants.

Yet another aspect of the present invention contemplates a method for generating male sterile plants, said method comprising transforming a cell or group of cells of said plant with a genetic construct capable of directing expression of a nucleotide sequence having a deleterious effect on tapetum and/or pollen tissue, regenerating a transgenic plant from said transformed cells and growing and/or maintaining said transgenic plant under conditions to thereby having a deleterious effect on said tapetum and/or pollen tissue resulting in said plant being substantially male sterile.

In an alternative embodiment, there is provided a method for generating male sterile plants, said method comprising introducing into a cell or group of cells of said plant, a genetic construct comprising all or part of a Bgp1, said Bgp1 having a nucleotide sequence substantially similar to an endogenous Bgp1 of the plant and then regenerating a plant from said cells. This method is term "co-suppression". The introduced Bgp1 may be with or without a promoter. By "substantially" similar is meant an exogenous Bgp1 comprising 85-100% nucleotide sequence similarity to an endogenous Bgp1.

Still yet another aspect of the present invention provides a transgenic male sterile plants such as those made by the above method.

The present invention is further described by the following non-limiting Figures and Examples:

Figure 1 is a schemmatic representation of the Bgp1 gene from *Brassica campestris*. **A.** Partial restriction map of genomic clone Bgp1. Box represents the sequenced region. **B.** Nucleotide sequence of Bgp1 (SEQ ID NO. 7) showing the coding region along with 5′ flanking region and 3′ flanking region; nucleotide numbering is relative to the start of the transcription at position number 1. The amino acid sequence of the putative Bgp1 protein is shown (SEQ ID NO. 2). The TATA box and the ATG translational start codon are underlined. Nucleotides which differ between the sequences of Bgp1 and Bcp1 are indicated by asterisks above the sequence.

Figure 2 is a photographic representation showing Bgp1 expression pattern in different organs of *B. campestris*. RNA transcripts are detectable only in pollen. Total RNA isolated from leaves, stems, flower minus anther and pollen was fractionated on a denatured agarose gel (20µg per lane), transferred onto a nylon membrane and probed with a Bgp1 specific oligonucleotide (based on the sequence between nucleotides 181-201; 5′-GGCTGCTACCGTAACCGATGT - 3′ [SEQ ID NO. 10]) labelled with ^{32}P. Bgp1 DNA and Bcp1 DNA were also probed to verify the specificity of the oligonucleotide, hybridisation was only observed to DNA from the clone Bgp1 as indicated.

Figure 3 is a photographic representation of DNA gel blot analysis of genomic DNA isolated from *B. campestris*. Genomic DNA was digested with the restriction endonucleases *Eco*RI, *Hin*dIII and *Bam*HI as indicated and probed with ^{32}P-labelled Bcp1 DNA. The position of *Hin*dIII digested 1 DNA is indicated.

Figure 4 is a photographic representation identifying the transcriptional start of the Bgp1 gene. Primer extension and plasmid sequencing (G, A, T, C) were performed using a synthetic oligonucleotide with sequence 5'-CGTTTTGGCGACCCA-3' (SEQ ID NO. 11) which is complementary to nucleotides 22-36. The nucleotide sequence at the 5' end is reported. The arrow indicates the position of the major extension product.

Figure 5 is a histochemical detection of GUS activity in transgenic *Arabidopsis* (A-H) and tobacco (I-J). GUS activity is indicated by blue staining (Jefferson *et al.*, 1986). A. mature flower showing the distributionof GUS activity. B, C. longitudinal section of a flower bud containing anthers at early bicellular stages, showing high level of GUS activity in tapetum (arrow heads). D. cross section of a near mature another showing GUS activity in degenerating tapetum (arrow heads) and pollen. E. cross section of an anther from control untransformed plants. F. cross section of a mature anther showing GUS activity in pollen, but not in other anther tissues. G. GUS staining in mature pollen. H. pollen of control untransformed plants. I. mature pollen of transgenic tobacco. J. mature pollen of control untransformed tobacco.

Figure 6 shows Bgp1 5' deletion fragments. Each of the fragments shown were fused to the GUS gene in the vector pBI101 and introduced into *Arabidopsis thaliana*. The full length promoter fragment pBC1.2 was also introduced into *Nicotiana tabacum*. Next to each promoter fragment is the GUS expression pattern observed for each corresponding pBI101 construct in *A. thaliana*.

Figure 7 is a working model showing the likely location of cis-acting DNA elements controlling the expression of the Bgp1 gene in pollen and tapetum. A+ indicates this region has a positive effect on expression and - signified a negative effect. The term min signifies the minimal promoter region necessary for pollen expression.

Figure 8 is a diagramatic representation showing Bgp1 antisense construct.

Figure 9 is a photographic representation of a comprison of *B. campestris* wild type (WT) and an antisense plant (AM) showing appearance of siliques.

Figure 10 is a photographic representation showing tissue-specific expression of the *Arabidopsis* Bgp1 gene. (a) RNA gel bot analysis showing the differential expression in vegetative and reproductive tissues. The transcripts of approximately 700 bp were detected in flowers, but not in the vegetative tissues tested. (b) *in situ* hybridisation of flower sections with biotin-labelled antisense (top panel) and sense (bottom panel) Bgp1-specific riboprobes. Flowers at two different developmental stages were used. RNA-RNA hybridisation signal was detected as bright regions on the sections. In immature flowers, an intensive hybridisation signal is present in the microspores (Mi) and the intact tapetal cells (Tc). In mature flowers, a very strong signal is present in pollen (Po) whereas only low level of signal is present in the remnants of tapetal cells (Tc) due to self-degeneration.

Total RNAs were isolated and used (15μg/lane) for the gel blot as described (Maniatis *et al.*, 1989). The blot was hybridised with a ^{32}P-labelled probe derived from *Brassica* Bcp1 cDNA clone. Flowers at relevant stages were collected, fixed and embedded in LR white resin for *in situ* hybridisation essentially as described (Theerakulpisut *et al.*, 1991). Biotin-labelled sense and antisense riboprobes were generated by *in vitro* transcription from the Bcp1 cDNA clone. Hybridisation signal was detected using colloidal gold (15 nm) conjugated rabbit anti-biotin antibody (1:15 dilution), followed by silver enhancement. Sections were viewed under dark field microscopy.

Figure 11 is a representation of (a) the nucleotide (SEQ ID NO. 8) and translated amino acid sequence (SEQ ID NO. 4) of the *Arabidopsis* Bgp1 gene. The sequence was determined by a genomic clone. The transcriptional initiation site determined by primter extension analysis was underlined (Xu, 1992). The longest open reading frame extends for 411 bp and translated into a 137 amino acids, approximately 14K protein with a highly hydrophobic region at N-terminus. A highly conserved (73% sequence identity) promoter region of 167 nucleotides which extends immediately

upstream from the transcriptional initiation site was shared by the homologous genomic clone, Bgp1 from *Brassica campestris*. Figure 11(b) is a diagram showing sequence identity between *Arabidopsis* Bgp1 gene and *Brassica* Bgp1 gene.

The genomic clone was isolated by screening an *Arabidopsis thaliana* ecotype Landsberg *erecta* genomic library using a probe derived from a homologous *Brassica* cDNA clone, Bcp1. DNA sequencing was performed by dideoxy chain termination method using T7 DNA sequencing kit (Pharmacia LKB). Specific oligonucleotide primers were used to obtain the complete sequence. Sequence analysis was performed using the Melbot/Angis.

Figure 12 is a photographic representation of DNA and RNA gel blot analysis of antisense male sterile plants. (a) Detection of antisense insertions in the primary antisense transformants (T_0). Genomic DNAs from wild type (WT) and 4 individual antisense male sterile plants were used for parallel hybridisations with a Bgp1 gene-specific probe (left panel) and a kanamycin-resistant gene, NPTII, specific probe (right panel). A 6.3 kb fragment (arrowhead) showed hybridisation with the Bgp-1 specific probe but not with NPTII probe indicating that it contains the endogenous Bgp1 gene. The insertions of antisense constructs in transgenic plants were confirmed by the presence of DNA fragments which hybridised with both Bgp1 and NPTII probes. (b) DNA gel blot analysis of 4 individual T_1 plants showing the ineritance of the antisense gene. (c) RNA gel blot analysis of 3 individual T_1 plants. The expression of endogenous and antisense Bgp1 gene in T_1 plants. The expression of endogenous and antisense Bgp1 gene in T_1 plants was determined using specific probes. The endogenous sense Bgp1 transcripts were detected in the flowers of control wild type (WT) untransformed plants, but not in any of T_1 plants. The expression of the antisense Bgp1 gene was detected in the flowers of male sterile T_1 plants, but not in the untransformed wild type plants.

Primary transformants (T_0) carrying antisense Bcp1 gene were cross-pollinated with wild-type to produce seeds. The T_1 progenies were grown in the greenhouse. Genomic DNA was extracted from leaf tissues of appropriate plants and digested with *Bam* H1, which does not cut inside the Bcp1 gene. DNA fragments were separated on 0.7% w/v agarose gel (10μg/lane) and transferred onto nylon membrane. The blots were hybridised with a ^{32}P-labelled probe derived from the Bcp1 cDNA clone. Parallel blots were hybridised with a NPTII gene probe. mRNA were isolated directly from flower inflorescences using Daneal Beads. RNA gel blots were prepared as described (Maniatis *et al.*, 1982). ^{32}P-Labelled sense and antisense riboprobes were generated by *in vitro* transcription from the Bcp1 cDNA clone.

Figure 13 is a photographic representation of male sterile (MS) *Arabidopsis thaliana*. (a) Flower inforescences from wild type (WT) plants. The plants produce elongated seed pods (Sp) resulted from self-pollination. Each seed pod yields 52-68 seeds. (b) Flower inflorescences from male sterile (MS) plants induced by transformation of the Bgp1 antisense gene. The male sterility is characterised by short and empty seed pods after self-pollination. (c) and (d) fluorochromatic reaction (FCR) test of pollen viability (Heslop-Harrison *et al* 1984). The viable pollen is characterised by the presence of bright fluorecence in pollen cytoplasm. Pollen grains from wild type plants showed 99% positive reaction (c), indicating high pollen viability, whereas pollen grains from male sterile plants gave no positive reaction (d), indicating that pollen is non-viable.

The Bcp1 antisense gene were constructed by inserting the 500 bp cDNA clone Bcp1 in the reversed orientation between an anther-specific promoter, Bgp1 and nonpaline synthase (nos) sequence. It was then cloned into a binary vector, Bin 19 (Bevan, 1984) and introduced into *Arabidopsis thaliana* (ecotype Landsberg *erecta*) using *Agrobacterium tumefaciens*-mediated transformation (Valvekens *et al.*, 1988). The transformants were selected on medium containing kanamycin. Pollen grains from both wild type and transformed plants were stained with fluorescein diacetate and viewed with fluorescnece microscopy under UV excitation (Heslop-Harrison *et al.*,

1984).

Figure 14 is a photographic representation showing differential staining of aborted and nonaborted pollen in nondehiscent anthers of male sterile plants using Alexander stain (Alexander, 1969). This stain differentially stains pollen walls (staining green) and pollen protoplasm (staining red). Anthers from wild type (WT) plants contain regular, spherical pollen grains with intensive red staining in the protoplasm. In strong contrast with the fertile pollen from wild type plants, the majority of pollen grains (>90%) from male sterile (MS) antisense primary transformants show only green staining of pollen walls indicating that the pollen grains are devoid of protoplasm and empty. The remaining grains had degenerated protoplasm as indicated by weak pink staining.

Figure 15 is a photographic representation showing light and electron microscopic analyses of mature anthers from male sterile plants showing the abnormalities of pollen grains. (a) Cross-sections of anthers from wild-type (WT) and male sterile antisense primary transformants (MS) shortly before dehiscencing. The majority of pollen grains in the anthers of male sterile plants has no internal protoplasm confirming the observation obtained by Alexander stain in Figure 14. (b) Transmission electron microscopic (TEM) studies of pollen from wild type and antisense male sterile plants. The male sterile pollen was completely empty and only the crushed exines are present. (c) Scanning electron microscopic (SEM) studies of fertile pollen from wild type plants and sterile pollen from antisense plants.

Mature flowers were fixed in 2% glutaradehyde and postfixed in 1% osmium tetraxide. After dehydration through an enthanol series, the flowers were embedded in Spur resin and sectioned. For light microscopy, semi-thin sections (1µm) were stained with toluidine blue and mounted. For TEM, ultra-thin sections were stained and viewed following standard procedure. For SEM observation, dehiscencing anthers were mounted on stab and air dried in a desiccator. The samples were observed after gold sputtering.

The following is a summary of the SEQ ID NOs referred to in the subject specification. The SEQ ID NOs are defined in full after the bibliography.

SUMMARY OF SEQ ID NOs

SEQ ID NO. 1	Open reading frame of *B. campestris* Bgp1
SEQ ID NO. 2	Deduced amino acid sequence of SEQ ID NO. 1
SEQ ID NO. 3	Open reading frame of *A. thaliana* Bgp1
SEQ ID NO. 4	Deduced amino acid sequence of SEQ ID NO. 3
SEQ ID NO. 5	Promoter region of *B. campestris* Bgp1
SEQ ID NO. 6	Promoter region of *A. thaliana* Bgp1
SEQ ID NO. 7	*B. campestris* Bgp1
SEQ ID NO. 8	*A. thaliana* Bgp1
SEQ ID NO. 9	*B. campestris* Bgp1 modified promoter -767 to -116
SEQ ID NO. 10	Bgp1 specific oligonucleotide
SEQ ID NO. 11	Bgp1 oligonucleotide
SEQ ID NO. 12	Bgp1 TATA box sequence
SEQ ID NO. 13	Consensus Bgp1 sequence

EXAMPLE 1

CLONING OF *BRASSICA* Bgp1

Construction of genomic library, screening and isolation of the genomic clone Bgp1. A genomic library was prepared from leaf material of *Brassica campestris* cv. T15. Genomic DNA was isolated according to standard procedures (Murray *et al.*, 1980) and partially digested with *Sau* 3A. *Sau* 3A fragments were size fractionated on a glycerol gradient (10-40%) by centrifugation at 40,000 rpm overnight. Aliquots of 500µl fractions were taken and diluted 1:2 in TE buffer (10mM TRIS-HCl,pH8.0; 1mM EDTA). DNA from the chosen fractions was then recovered by precipitation with ethanol and centrifugation at 13,000g for 30 minutes. The resultant 9-23kb

fragments were ligated into EMBL3 *Bam* H1 arms (Stratagene). The ligation mix was then packaged into phage using Packagene (Promega Biotec.) to yield the genomic library. The library was plated on LB media at a density of approximately 10 000 plaques per 90mm plate using *Escherichia coli* NW2 (Woodcock *et al.*, 1988) as the host strain. Duplicate plaque lifts were performed using Hybond-C extra following the manufacturer's protocol. The filters were hybridized with ^{32}P-labelled Bgp1 in 2xSSPE, 0.5% w/v Blotto, 1% w/v PEG 20 000, 7% w/v SDS and 250 mg/ml (final volume) denatured Herring sperm DNA at 65 °C. Filters were washed at 65 °C for 30 minutes in 2xSSC, 0.1% w/v SDS and for 15 minutes in 0.2xSSC, 0.1% w/v SDS. Filters were exposed to Kodak X-Omat film overnight at -70 °C. The genomic clone obtained is designated "Bgp1".

DNA Sequencing.

A series of overlapping deletion clones (Bgp1.1-Bgp1.7) were generated from the Bgp1 4.2 kb *Hin*dIII genomic fragment by digestion with Exonuclease III and religation. The protocols supplied with the Nested Deletions kit (Pharmacia LKB) were followed. Southern blot analysis demonstrated that Bgp1.1 - Bgp1.5 but not Bgp1.6 and Bgp1.7 show homology to Bgp1. Sequencing then commenced using Bgp1.1 though Bgp1.5 as templates. Sequencing reaction was performed on double-stranded template according to the T7 polymerase sequencing kit manual (Pharmacia LKB). Both strands were sequenced using T7, SP6 or synthetic primers made to internal sequences.

RNA and DNA gel blot analyses.

RNA gel blot hybridizations were performed using total RNA (20µg per lane) separated by electrophoresis on formaldehyde-agarose gels and blotted onto Hybond-N (Amersham) nylon filters (Maniatis *et al.*, 1982). Filters were prehybridized, hybridized with ^{32}P-labelled oligonucleotide and washed according to the manufacturers specifications (Amersham). DNA gel blots were performed using 10µg of *B.campestris* DNA per digest separated on 0.8% w/v agarose gels and blotted onto Hybond-N filters following standard protocols (Maniatis *et al.*, 1982). Prehybridization, hybridization with ^{32}P-labelled DNA and washing was again done

according to the manufacturer's specifications (Amersham).

Primer extension analysis.

The transcriptional start point of Bgp1 was determined by primer extension analysis performed according to standing procedures (Maniatis *et al.*, 1982). A 15-mer synthetic oligonucleotide of sequence 5'-CGTTTTGGCGACCCA-3' (SEQ ID NO. 11) complementary to nucleotides 22 - 36 of Bgp1 was end-labelled with [$g^{32}P$] ATP (Amersham) and T4 polynucleotide kinase (Promega Biotech). After annealing and extension of the primer, the products were analysed on a polyacrylamide sequencing gel.

Construction of plasmids.

The 0.8 kb P*st* I-*Hae* III DNA fragment (from position - 767 to +100 including the 0.7 kb 5' flanking region and 100 nucleotides of the 5' untranslated leader sequence of the Bgp1 gene), was excised from Bgp1.3, a deletion clone of Bgp1, and ligated to Bluescript (+) KS (Stratagene). This fragment was then excised as a *Hin*dIII-*Bam* HI fragment and inserted into the polylinker of the vector, pBI 101 (Stratagene). This vector is a derivative of the binary vector pBIN 19 (Bevan, 1984) which contains a promoter-less *gus* gene cassette (Jefferson *et al.*, 1986) fused to the nopaline synthase polyadenylation region. The resulting Bgp1-GUS chimeric construct, designated as pBgp1.2, was mobilized into *Agrobacterium tumefaciens* strain LBA 4404, by conjugating with a helper plasmid pRK 2013 (Koncz and Schell, 1986).

Plant transformation.

(i) Tobacco: leaf discs of *Nicotiana Tabacum var.* Wisconsin 39 were transformed with *Agrobacterium tumefaciens* essentially as described in Horsch *et al.* (1985). Shoots arising from leaf discs were rooted on MS medium (Gibco Laboratory) containing 1.0 µg/ml IAA, 1.0 µg/ml BAP, 100 µg/mlKanamycin, transferred to soil and grown to flowering in the greenhouse. *Arabidopsis thaliana var* Landsberg roots were transformed according to Valvekens *et al.* (1985). Transgenic plants were selected on medium containing 50 µg ml^{-1} kanamycin.

GUS assay.

Histochemical GUS assays were performed essentially as described by Jefferson *et al.* (1987). For histochemical assay, plant materials were placed in the wells of a microtiter plate containing 1 mM X-Glu (5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid, Sigma) in 0.1 M $NaPO_4$pH 7.0, 0.1% v/v Triton-X 100 and incubated at 37 °C for 8 to 12 h. In *Arabidopsis*, the positively stained flowers were processed for cryo-sectioning. The samples were embedded, rapidly frozen in Tissue-Tek OCT compound (Miles Inc., Elkhart, IN) and sectioned at -20 °C using a 2800 Frigocut cryostat (Reicher-Jung, Germany). The developmental stages of anthers were determined by staining the sections with DAPI (Coleman and Goff, 1984).

Genomic clone Bgp1 is highly homologous to the cDNA clone Bcp1.

A genomic clone, designated Bgp1 was isolated from *Brassica campestris* using cDNA clone Bcp1 as a probe. A partial restriction endonuclease site analysis of the 11kb genomic fragment in Bgp1 resulted in the map presented in Figure 1. Southern blot analysis using Bcp1 as a probe revealed that a 4.2kb Hind III fragment from the 11kb genomic clone contained the coding region of the gene. This fragment was then subcloned and partially sequenced.

A total of 1620bp of DNA was determined, this included the entire sequence of the coding region along with 767bp of 5′ flanking sequence. Comparison of the cDNA sequence of Bcp1 (Theerakulpisut *et al.*, 1991) with the coding region of genomic clone Bgp1 revealed an overall homology of 88%. No introns are present. The sequence of the Bgp1 coding region, 767bp of 5′ flanking region and 392bp of 3′ flanking region is presented in Figure 1.

EXAMPLE 2

TISSUE-SPECIFIC EXPRESSION OF Bgp1 ENDOGENOUS GENE

In order to determine the expression pattern of Bgp1 gene, a Bgp1-specific oligonucleotide was synthesized based on the sequence between nucleotides 181 - 201 (5′ - GGCTGCTACCGTAACCGATGT - 3′ [SEQ ID NO. 10]) (Fig. 1), a region which shows a high level of variability between the two genes, Bgp1 and Bcp1. This 21mer oligonucleotide was used to probe a Northern blot containing total RNA isolated from *B. campestris* pollen, leaf, stem, and flower (minus anther). As a negative control Bcp1 DNA was included on the blot to ensure the specificity of the oligonucleotide. Figure 2 shows that the Bgp1-specific oligonucleotide hybridizes to RNA present in pollen but not to RNA present from any other tissue tested. The size of the transcript = 700 nucleotides is approximately the same size as the RNA transcript to which clone Bcp1 hybridizes (Theerakulpisut *et al.*, 1991).

EXAMPLE 3

Bgp1 BELONGS TO A SMALL GENE FAMILY

To determine whether the clone Bcp1 represents a transcript from a member of a gene family, the cDNA insert was used to probe a DNA gel blot of total *B.campestris* DNA. Figure 3 shows that Bcp1 cDNA insert hybridizes to several genomic bands including the 4.2kb *Hind* III fragment representing the Bgp1 gene. It is difficult to estimate the gene family copy number from this blot but there are at least two members in the gene family.

EXAMPLE 4

DETERMINING THE TRANSCRIPTIONAL START OF Bgp1

The transcriptional start point of Bgp1 was determined by primer extension analysis. An oligonucleotide was synthesized based on the sequence between nucleotides 22 - 36 (Fig. 1). Figure 4 shows that when this primer was used in extension analysis and the labelled products run next to the sequence of clone Bgp1, a fragment of length 61 nucleotides can be detected. This indicates that the A nucleotide at position 1 (Fig. 1) is the first nucleotide transcribed from the Bgp1 gene. Fainter bands are likely to be due to homologous transcripts from other members of the Bcp1 gene family.

EXAMPLE 5

SEQUENCE ANALYSIS

The promoter region of the Bgp1 gene contains a TATA box sequence (CAACTATATATAG [SEQ ID NO. 12]) which is located 26 nucleotides upstream of the start of transcription (see Fig. 1). In agreement with the "scanning mechanism" theory (Kozak, 1986), the translational start of the Bgp1 gene is the first ATG codon present in the sequence, which is found 69 nucleotides downstream from the start of transcription (Fig. 1). The putative start codon gives the longest possible open reading frame (357 nucleotides) and its flanking regions match perfectly with the consensus proposed for plant genes of AACAATGGC [SEQ ID NO. 13] (Lutcke *et al.*, 1987). The resulting untranslated leader sequence is characteristically A/T rich (63%).

The predicted protein sequence of clone Bgp1 is shown under the nucleotide sequence in Fig. 1. The Bgp1 protein sequence is very similar to the predicted protein sequence from Bcp1 (Theerakulpisut *et al.*, 1991) showing 87.5% identity. A computer search comparing the nucleotide and amino acid sequence of clone Bgp1 with the sequences contained in GenBank, EMBL and NBRF databases revealed no

significant homologies.

The 5' upstream region of clone Bgp1 was examined for homology to the promoter regions of both anther and pollen specific genomic clones. A comparison of the entire 767bp Bgp1 5' region was made with a range of 5' sequences from pollen/anther specific genes (Hamilton *et al.*, 1989; Twell *et al.*, 1989, 1991; Koltunow *et al.*, 1990; Albani *et al.*, 1991a, 1991b). No significant regions of homology were observed.

EXAMPLE 6

TRANSFORMATION OF THE FULL LENGTH PROMOTER CONSTRUCT INTO *ARABIDOPSIS* - HIGH LEVELS OF GUS ACTIVITY PRESENT IN POLLEN AND TAPETUM

The histochemical distribution of the GUS activity driven by the 767bp Bgp1 5' region carried by the construct pCB1.2 is illustrated in Figure 5. Figure 5A shows that high levels of GUS activity were present in anthers, but not in petals, sepals, filaments and pistils. No GUS activity was detected in anthers of control untransformed plants.

The developmental pattern of Bgp1-promoter activity was also analysed in transgenic plants carrying the construct pCB1.2. In cryosections of developing *Arabidopsis* anthers containing an intact tapetum, Figures 5B and 5C show that high levels of GUS activity were present in the tapetum, whereas only low levels were detactable histochemically in the pollen at early bicellular stage. In near mature anthers, in which the tapetum had begun to degenerate, Figure 5D shows that high levels of GUS activity were present both in the degenerating tapetum and pollen grains. Figures 5F and 5G show that very high levels of GUS activity were present in mature pollen, but not in other tissues of the anther. Figures 5E and 5H show that tissues of control untransformed anthers and pollen produced no histochemically detectable levels of GUS activity.

EXAMPLE 7

TRANSGENIC TOBACCO PLANTS SHOW GUS ACTIVITY IN POLLEN ONLY

In transgenic tobacco plants carrying pCB.2, GUS activity was detected in pollen (Figure 5I). In addition GUS activity was tested in anther sections taken from flower buds at several developmental stages. In tobacco, flower bud length correlates well with gametophytic development (Koltunow *et al.*, 1990). Sections were taken from flower buds of sizes 3mm (tapetum formation commences), 4mm (tapetum and pollen sacs distinct), 5mm (meiosis begins), 6mm (tapetum large and multinucleate), 7mm, 8mm (meiosis complete) through to 14mm (tapetum shrunken, pollen grains begin to form). No GUS activity was detected in the tapetum at any of these developmental stages.

EXAMPLE 8

ANALYSIS OF BGP1 5' PROMOTER DELETIONS

To identify *cis*-acting elements controlling the temporal and spatial expression pattern of Bgp1 a series of 5' deletion clones were created. These constructs, shown in Figure 6, were transferred to *Arabidopsis thaliana by Agrobacterium tumefaciens*-mediated transformation. GUS activity was analysed on primary transformants. At least 10 individual transformants were analysed for each construct. The GUS expression pattern for each of the constructs is presented alongside each of Figure 6.

Deletion of the full length promoter down to -580 (pCB1.3) abolished any detectable GUS expression in the pollen of 87% of the plants tested. Expression in the tapetum of plants carrying pCB1.3 was unaffected. However, if further deletion removed the region between -322 and -580 (pCB1.4), GUS expression in the pollen was restored in all the plants tested. Progressive 5' deletions down to -260 (pCB1.5) and -168 (pCB1.6) gave the same result, GUS expression was observed in both the tapetum and the pollen. The smallest construct tested however, which contained only the 5' region up to position -116, directed GUS expression in the tapetum only.

EXAMPLE 9

INDUCING MALE STERILITY IN *ARABIDOPSIS* BY ANTISENSE

Construction of antisense gene

A Bcp1 antisense gene was constructed by inserting the cDNA clone Bcp1 in the reverse orientation between an anther-specific promoter, Bgp1 and nopaline synthase (nos) sequence. It was then cloned into the plant transformation vector, Bin 19 (Figure 8). The resulting construct was mobilised to *Agrobacterium tumefacien* strain LBA 4404 and introduced into *Arabidopsis thaliana var* Landsberg using standard procedures (Valvekens *et al*, 1988). The transgenic plants carrying the antisense construct were selected by Kanamycin resistance.

Phenotype modification of the transgenic plants carrying Bcp1 antisense construct

The transgenic plants were examined for male-fertility in terms of the number of seeds produced following self-pollination. A total of 50 flowers from each of 20 different healthy plants were examined. All the plants produce phenotypically normal flowers. However, the plant produced short siliques typical of male sterile plants (Moffat and Sommerville, 1988) and no seeds were set after selfing (Figure 9).

Female-fertility of the transgenic plants

The transgenic plants carrying the Bcp1 antisense construct were examined for their female-fertility by cross-pollinating with pollen from Wild-type plants. Ten flowers from three transgenic plants were pollinated with Wild-type pollen. All the flowers produced normal elongated siliques following cross-pollination, indicating that female function is normal in these antisense transgenic plants.

Pollen morphology in Antisense plants

Pollen grains from both Wild-type and antisense plants were examined by scanning electron microscopy for possible alteration of pollen surface structure and morphology. Pollen from five of the antisense plants appeared to be collapsed and shrunken when prepared a similar way to wild-type pollen. Some of the pollen

grains showed aberrant exine structure. In one antisense plant, the formation of the fish-net patterned ektexine was irregular, with prominent patches where the ektexine was missing over the pollen surface.

In the antisense plants, light and transmission electron microscopic analyses showed that in mature pollen, the internal protoplasmic structure was completely disorganised or empty. Developmental studies showed that tapetal and microspore differentiation was normal until the time of first pollen mitosis. The cytoplasm of the pollen grains then developed mutiple vacuoles, and became disorganised. These data indicate that sterility of the pollen grains sets in at about the time of maximal expression of the gene Bcp1 in the pollen grains. While tapetal development appeared normal, the 100% effectiveness of the antisense construct in all 20 plants suggests that expression of Bgp1 in the tapetum is vital for normal pollen development.

Pollen viability test (FCR test)

Pollen grains from Wild-type and antisense plants were examined for viability using the Fluorochromatic Reaction (FCR) test (Heslop-Harrison *et al.*, 1984). Pollen from Wild-type gave 99% positive reaction, indicating high pollen quality, whereas pollen from antisense plants showed no positive FCR staining, indicating that pollen quality has been lost, and membrane integrity has been detrimentally altered.

These data show that Bgp1 gene is essential for normal pollen development. This is shown by the male sterility induced when the gene is present in antisense RNA version. Bgp1 is expressed in both the tapetum and pollen, and down regulation of its expression in the antisense plants clearly shows the importance of the gene product for normal development.

EXAMPLE 10

CLONING HOMOLOGOUS GENE FROM ARABIDOPSIS

RNA gel blot studies indicated that a gene homologous to *B. campestris* Bgp1 is expressed in *Arabidopsis thaliana* (Figure 10). The specificity and pattern of expression in anthers of *Arabidopsis* Bgp1 was isolated by screening an *Arabidopsis* genomic library with the *Brassica* Bgp1 cDNA clone (Figure 11). DNA sequencing studies show that the *Arabidopsis* Bgp1 cDNA genomic clone in 1132 bp, with an ORF of 137 amino acids (compared with 119 in *Brassica*). The deduced amino acid sequence does not contain introns and encodes an alanine-rich (16%) protein with a relative molecular mass, M_r 14K (compared with 12K *Brassica*). The nucleotide and deduced amino acid sequences of Bgp1 show no homology with other known genes or proteins in the databases. No potential N-glycosylation sites are present in the amino acid sequence. Mouse polyclonal antibodies raised against two synthetic peptides based on hydrophylic regions of the *Brassica* Bgp1 amino acid sequence recognised M_r 11-12 K polypeptides by Western analysis. Accordingly, the results indicated that the Bgp1 gene is expressed specifically in tapetum and pollen and encodes a protein of M_r12-14K in both *Brassica* species and *Arabidopsis* species.

EXAMPLE 11

CONTROL OF ANTHER-SPECIFIC EXPRESSION OF ARABIDOPSIS Bgp1

To demonstrate that 5′ sequences control Bgp1 gene developmental specificity, the *Escherichia coli* GUS gene was fused with a 0.77 kb upstream fragment (nucleotides -767 to +100; Xu *et al.*, 1993), containing the start codon and then transformed *Arabidopsis* plants with the chimaeric Bgp1 GUS gene. Several independent transformants were obtained. Each transformant showed GUS enzyme activity in both tapetum and pollen. The pattern of GUS activity in anthers of transgenic plants is consistent with the expression of endogenous Bgp1 gene in *Brassica* and *Arabidopsis*.

Comparison of 5'-flanking regions of Bgp1 from both *Brassica* and *Arabidopsis* shows that the two genes share a conserved region of high homology in the 167 nucleotides that lie immediately upstream of the transcriptional initiation site (Figure 11b). There is no significant homology between the 5' regions of the two genes beyond this point. Because of the highly conserved pattern of expression of this gene in anthers of the two genera, it was expected that this 167 bp 5' region may be sufficient to direct the normal developmental expression of the genes. To examine this, a chimaeric gene was constructed by fusing the 167 bp fragment with GUS (nucleotides -167 to +100; Xu *et al.*, 1993). The *Arabidopsis* plants transformed with this construct showed the same pattern of GUS enzyme activity in anthers as those transformed with larger promoter fragments. Since GUS enzyme activity in plants transformed with a truncated 5' fragment appeared to be relatively less than those with the larger fragment, it was decided that the region upstream of the -167 bp may have an enhancer effect for Bgp1 gene expression.

EXAMPLE 12

ANTISENSE INHIBITION OF Bgp1 GENE EXPRESSION INDUCES MALE STERILITY

The 0.77 kb Bgp1 gene regulatory fragment was fused with antisense Bcp1 cDNA expression. This chimaeric construct was introduced into *Arabidopsis* plants and 22 primary transformants (T_0) were obtained. The transformants appeared identical to untransformed control plants with respect to growth rate, height, leaf and flower morphology, time of flowering and flower colour (Figure 13). However, 7 of antisense transformants failed to show elongation of siliques, indicating loss of fertility.

Microscopic examination of flowers of antisense transformants (T_0) showed the presence of defective pollen grains in the anthers, confirming that the effect is specifically on male rather than female fertility. Pollen from anthers of antisense transformants was negative when tested for pollen quality by FCR test (Heslop-Harrison *et al.*, 1984) compared with pollen from anthers of normal plants (Figure

13). Use of Alexander's stain (which indicates the presence or absence of cytoplasm in pollen grains as a measure of sterility) showed that >90% of pollen in antisense transformants is present as empty exines (green staining), while the remaining grains had cytoplasm (weak pink or red staining) in various stages of degeneration (Figure 14). In contrast, pollen from anthers of normal plants showed densely staining (purple) grains.

Sections of anthers were prepared from both transformed and untransformed (normal) plants. Male sterile anthers showed collapsed pollen sacs, and pollen grains without visible cytoplasmic contents (Figure 15a). Rare grains showed some residual cytoplasm that appeared disorganised and lysed (Figure 15a). All other anther tissues and cell types appeared identical to normal anthers.

DNA gel blot analysis of the male sterile primary transformants showed that the male sterility phenotype is linked with the presence of the antisense cDNA in their genome (Figure 12a). The presence of the antisense insert was tested both by use of Bgp1 cDNA and neomycin phosphotransferase (NPTII) as hybridisation probes.

To determine whether the male sterility is a stably inherited trait, antisense transformants were crossed with pollen from normal (untransformed) plants. Normal silique formation and seed set occurred in all cases. These results indicate that antisense transformants are male sterile, their pistils are able to recognise and transmit pollen normally, and female fertility is unaffected. Eight of T_1 plants were analysed and all inherited the male sterility phenotype. In 4 of 8 T_1 plants, the presence of antisense Bgp1 gene was further analysed by DNA gel blot analysis (Figure 12b). The male sterile phenotype and presence of antisense insert completely co-segregated. The introduced gene is present in the genomic DNA of the analysed T_1 plants with male sterile pollen and absent in the genome of T_1 plants with normal viable pollen. Inheritance of male sterility phenotype is also observed in T_2 generation.

The expression of both endogenous and antisense mRNA expressing Bgp1 in both primary transformants was studied and male sterile T_1 plants (Figure 12c). With sense-specific probe, a single mRNA band of approximately 700 bp was detected in flowers of untransformed plants. The presence of sense Bgp1 mRNA could not be detected in either primary transformed or T_1 plants with male sterility phenotype. However, using an antisense-specific probe, a strongly hybridising transcript of approximately 750 bp was detected in flowers of all male sterile transformants. No antisense RNA was detected in control untransformed plants. Thus, the male sterility phenotype is linked with high expression of antisense Bcp1 mRNA and loss of sense Bcp1 mRNA.

EXAMPLE 13

ANTISENSE TRANSFORMANTS SHOW PROGRAMMED CELLULAR AUTOLYSIS DURING POLLEN DEVELOPMENT

In order to define the stage of pollen development when arrest is initiated, thin sections of developing anthers of both normal plants and antisense transformants were prepared. At tetrad and uninucleate microspore stage, both tapetal cells and microspores appeared normal in both types of anthers. This is the stage when tapetum is most active and the exine is completely formed. At the late microspore stage, the microspore cytoplasm showed signs of vacuolation and autolysis (Figure 14) which appeared to be complete before microspore mitosis. This rapid loss of cellular contents ultimately results in complete collapse of the microspores, which appear as empty shells (Figure 15), since the exine remains unaffected. The tapetum appeared normal in both types of anther. This developmental sequence was similar in both primary transformants (T_0) and male sterile T_1 generation.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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SEQUENCE LISTING

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(ii) TITLE OF INVENTION: DEVELOPMENTAL REGULATION IN ANTHER TISSUE OF PLANTS

(iii) NUMBER OF SEQUENCES: 13

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
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(B) FILING DATE: 15-DEC-1993

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 357 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..357

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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ATG GGT CGC CAA AAC GCT GTC GTA GTT TTT GGC CTT GTG TTC TTG GCC        48
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 1               5                  10                  15

ATC CTT GGC CTC GCC GCA GCT GCC TCC TCT CCG TCT CCT TCA GCG TCA        96
Ile Leu Gly Leu Ala Ala Ala Ala Ser Ser Pro Ser Pro Ser Ala Ser
            20                  25                  30

CCC TCC AAA GCT CCG GCT GCT ACC GTA ACC GAT GTC GAA GCT CCA GTG       144
Pro Ser Lys Ala Pro Ala Ala Thr Val Thr Asp Val Glu Ala Pro Val
        35                  40                  45

AGC GAG GAC ACC ATT GGA ACC ACC GAT GAC GAT GCA GCT GCT TCT CCA       192
Ser Glu Asp Thr Ile Gly Thr Thr Asp Asp Asp Ala Ala Ala Ser Pro
    50                  55                  60

GGT GAT GGT GAC GTA GCT GTG GCT GGT CCT CTA GGA AGT GAC TCC TCC       240
Gly Asp Gly Asp Val Ala Val Ala Gly Pro Leu Gly Ser Asp Ser Ser
65                  70                  75                  80

TAC GGT AGT AAT GGA CCT TCA CCT TCT ACT GAT GCT GCT GAC AGC GGC       288
Tyr Gly Ser Asn Gly Pro Ser Pro Ser Thr Asp Ala Ala Asp Ser Gly
                85                  90                  95

GCG CCT GCT CTT GGC GTC TCT GCG GTC TTC GTT GGT GTT GCA TCC ATC       336
Ala Pro Ala Leu Gly Val Ser Ala Val Phe Val Gly Val Ala Ser Ile
            100                 105                 110

GCC GGT TCT TTC TTG TTT CTC                                          357
Ala Gly Ser Phe Leu Phe Leu
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Gly Arg Gln Asn Ala Val Val Val Phe Gly Leu Val Phe Leu Ala
1 5 10 15

Ile Leu Gly Leu Ala Ala Ala Ala Ser Ser Pro Ser Pro Ser Ala Ser
20 25 30

Pro Ser Lys Ala Pro Ala Ala Thr Val Thr Asp Val Glu Ala Pro Val
35 40 45

Ser Glu Asp Thr Ile Gly Thr Thr Asp Asp Asp Ala Ala Ala Ser Pro
50 55 60

Gly Asp Gly Asp Val Ala Val Ala Gly Pro Leu Gly Ser Asp Ser Ser
65 70 75 80

Tyr Gly Ser Asn Gly Pro Ser Pro Ser Thr Asp Ala Ala Asp Ser Gly
85 90 95

Ala Pro Ala Leu Gly Val Ser Ala Val Phe Val Gly Val Ala Ser Ile
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Ala Gly Ser Phe Leu Phe Leu
115

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 411 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..411

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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Met Gly Arg Gln Asn Ile Val Val Val Val Ala Leu Val Phe Ile Arg
 1               5                  10                  15

ATC ATT GGC CTT GCC GCA GCT GCC TCC TCT CCA TCT CCT TCA GCG TCT      96
Ile Ile Gly Leu Ala Ala Ala Ala Ser Ser Pro Ser Pro Ser Ala Ser
            20                  25                  30

CCC TCC AAA GCT CCA GCT GCC TCC AAA ACC GAT CAT GTC GAG GCT CCA     144
Pro Ser Lys Ala Pro Ala Ala Ser Lys Thr Asp His Val Glu Ala Pro
        35                  40                  45

GTC ACC GAT GAC CAA ATC GGA ACC ACC GAT GAC GAT GCA GCT CCT ACT     192
Val Thr Asp Asp Gln Ile Gly Thr Thr Asp Asp Asp Ala Ala Pro Thr
    50                  55                  60

CCT GGT GAC GGT GAC GTT GCA GTG GCT GGT CCT CTA GGA AGT GAC TCC     240
Pro Gly Asp Gly Asp Val Ala Val Ala Gly Pro Leu Gly Ser Asp Ser
65                  70                  75                  80

TCG TAC GAC AAT GCC GCT ACA GGC TCT GCT GAT TCT GCC AAA AGC GGT     288
Ser Tyr Asp Asn Ala Ala Thr Gly Ser Ala Asp Ser Ala Lys Ser Gly
                85                  90                  95

GCG GCA GCT CTT GGC GTC TCT GCG GTC GTC GTT GGT GTT ACA TCA TTG     336
Ala Ala Ala Leu Gly Val Ser Ala Val Val Val Gly Val Thr Ser Leu
            100                 105                 110

CTG GTT CTT TCT TGT TAC TCA AGT TGG GCA TTG TTT TAT GAT AAG AAG     384
Leu Val Leu Ser Cys Tyr Ser Ser Trp Ala Leu Phe Tyr Asp Lys Lys
        115                 120                 125

GTT ATT TTA AAC GAA GAT TAT TAT ATG                                411
Val Ile Leu Asn Glu Asp Tyr Tyr Met
    130                 135
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 137 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Gly Arg Gln Asn Ile Val Val Val Val Ala Leu Val Phe Ile Arg
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Ile Ile Gly Leu Ala Ala Ala Ala Ser Ser Pro Ser Pro Ser Ala Ser
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Pro Ser Lys Ala Pro Ala Ala Ser Lys Thr Asp His Val Glu Ala Pro
35 40 45

Val Thr Asp Asp Gln Ile Gly Thr Thr Asp Asp Asp Ala Ala Pro Thr
50 55 60

Pro Gly Asp Gly Asp Val Ala Val Ala Gly Pro Leu Gly Ser Asp Ser
65 70 75 80

Ser Tyr Asp Asn Ala Ala Thr Gly Ser Ala Asp Ser Ala Lys Ser Gly
85 90 95

Ala Ala Ala Leu Gly Val Ser Ala Val Val Val Gly Val Thr Ser Leu
100 105 110

Leu Val Leu Ser Cys Tyr Ser Ser Trp Ala Leu Phe Tyr Asp Lys Lys
115 120 125

Val Ile Leu Asn Glu Asp Tyr Tyr Met
130 135

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 838 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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ATTGTTTCTT TAATTGTTAC CAATTAGAAC TTTAACGAAT AAATAGTTAA TTCGTATTAT 180

GAGATTTACA CAATTCTTAT TCACTCAATT TGGAGTTTTA AAGATTTTTT AAAAGATTTA 240

TGGTGGGAAC CTTCTTCTTT TCTTATTTAT CATGATGATG ATAACCTTCC CAGCAGAATT 300

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TGTATGCATT CGTTTTAGCC TGTAAGATAT CAGACATTCA CGTTTCGATA AACAAGTATA 480

TAAATAATAT GAATATTGTA CATTCATTTT ATTCGGTTCA TCAACCAAAA AAAATAAAAA 540

TAAATATTCG TATTCATCTA TGCTTTGGCA TGGTCCGTTC TTTTTTCTTG ATTGGCTCGT 600

TACCATTCAA AAATATATAC CTTAGCAAAC CCATTTTTAG ACATTCCAGT TGATCTACAT 660

TAGATTGAAC GGTATTCCTC CTACGTAGTA AGAACGTTTT CTATTTTTCT TTGTTTCAGT 720

CATACAACAC AACTATATAT ACACAGCAAC CCCATCTCCT CTCCAATCAT CACAATCTCT 780

AACGTTAAAC CCTAAGACAA ACTAAAAGAG AGCTACGTAC AAGGAGACAG AGAGAAGA 838

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 496 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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TAACTCGCTG TTGATGGCAG AATCGTAAAT CGGAATTGAT TCATGGGCCT AACAAGACGT 120

TTGGGCTTAT GGGTTTAAAG CCCATCTGAT ATAAGATGAA TAGAATGTTC ATGGCAATAC 180

TATCATAATT TGGTTCTTTA ATAAGACACT CGTTAATACG ACGACGATTT GAAGTTGAAC 240

GAATGTTTTC ATATTCATTC GCATGTTCAC CAATCAAAAT CTATATCTGA ACAAGTCCAT 300

TTTTAGGTAC TCCAGTAGAT TTACATTGGA TTGTAAGGTA ATCCTACATC TTAGTTCACG 360

TTTTCTATTT TTGGTCTTGT CACTAAACAC AACTATATAT ACATATCAAA CTCATCTTCG 420

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(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1621 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

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ATTGTTTCTT TAATTGTTAC CAATTAGAAC TTTAACGAAT AAATAGTTAA TTCGTATTAT 180

GAGATTTACA CAATTCTTAT TCACTCAATT TGGAGTTTTA AAGATTTTTT AAAAGATTTA 240

TGGTGGGAAC CTTCTTCTTT TCTTATTTAT CATGATGATG ATAACCTTCC CAGCAGAATT 300

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(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1132 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

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GAATGTTTTC ATATTCATTC GCATGTTCAC CAATCAAAAT CTATATCTGA ACAAGTCCAT 300

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GAAATCATCA CAATCAATAA ACCTCAAACC CTAAAATAAA TTAAACGAGT TCTACGTAAG 480

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CCGGATCATT GGCCTTGCCG CAGCTGCCTC CTCTCCATCT CCTTCAGCGT CTCCCTCCAA 600

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AACCACCGAT GACGATGCAG CTCCTACTCC TGGTGACGGT GACGTTGCAG TGGCTGGTCC 720

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TTATATGTAA GGATGATTGT GATGATCCGT TGACCTGCAG GTCGACCCAG ATCCGCCTAC 960

CTTTCACGAG TTGCGCAGTT TGTCTGCAAG ACTCTATGAG AAGCTGATAA GAGATAAGTT 1020

TGCTCAACAT CTTCTCGGGC ATAAGTCCGG ACACCATGGC ATCACAGTAT CGAGATGACA 1080

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(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 652 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TATCATTCCT TTAATTTCAA GGAATTATAG AACAAAAAAT GTTCTTTATA AAAATTAAGA 60

AGGAACAAGG GATTCATTCC TACTATTCTG TTCTTGGTCA TTATTTTCCT CTTCATTCAT 120

ATTGTTTCTT TAATTGTTAC CAATTAGAAC TTTAACGAAT AAATAGTTAA TTCGTATTAT 180

GAGATTTACA CAATTCTTAT TCACTCAATT TGGAGTTTTA AAGATTTTTT AAAAGATTTA 240

TGGTGGGAAC CTTCTTCTTT TCTTATTTAT CATGATGATG ATAACCTTCC CAGCAGAATT 300

ATTCTTAGAA CTTTTTTTCA CATTTAGGTA TCCATGCCTA AGTAAGGCTT AGTTAAAGAT 360

GTTTTATAAA CTTTGATCAA AATATTCATT CAATTAATTT GAGCTTCAAC TATAAATTGT 420

TGTATGCATT CGTTTTAGCC TGTAAGATAT CAGACATTCA CGTTTCGATA AACAAGTATA 480

TAAATAATAT GAATATTGTA CATTCATTTT ATTCGGTTCA TCAACCAAAA AAAATAAAAA 540

TAAATATTCG TATTCATCTA TGCTTTGGCA TGGTCCGTTC TTTTTTCTTG ATTGGCTCGT 600

TACCATTCAA AAATATATAC CTTAGCAAAC CCATTTTTAG ACATTCCAGT TG 652

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGCTGCTACC GTAACCGATG T 21

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CGTTTTGGCG ACCCA 15

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CAACTATATA TAG 13

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AACAATGGC 9

CLAIMS:

1. A genomic DNA isolate comprising:
   (i) all or part of a gene or related genetic sequence preferentially expressed in anther tissue of a plant and substantially not expressed in non-anther tissue; and
   (ii) an open reading frame having a nucleotide sequence as set forth in SEQ ID NO. 1 or having at least 20% similarity thereto.

2. A genomic DNA isolate according to claim 1 wherein the plant is a dicotyledonous plant.

3. A genomic DNA isolate according to claim 2 wherein the plant is a *Brassica* species, *Arabidopsis* species or *Nicotiana* species.

4. A genomic DNA isolate according to claim 3 wherein the plant is *Brassica campestris* and the nucleotide sequence of its open reading frame is as set forth in SEQ ID NO. 1.

5. A genomic DNA isolate according to claim 3 wherein the plant is *Arabidopsis thaliana* and the nucleotide sequence of its open reading frame is as set forth in SEQ ID NO. 2.

6. A genomic DNA isolate according to claim 1 further comprising a promoter region 5′ to the open reading frame, wherein said promoter region:
   (i) is capable of directing expression in tapetum and/or pollen tissue; and
   (ii) comprises a nucleotide sequence as set forth in SEQ ID NO. 3 or having at least 20% similarity to all or part thereof.

7. A genomic DNA isolate according to claim 6 comprising a nucleotide sequence as set forth in SEQ ID NO. 3.

8. A genomic DNA isolate according to claim 6 comprising a nucleotide sequence as set forth in SEQ ID NO. 4.

9. A genomic DNA isolate comprising:

(i) all or part of a gene or related genetic sequence preferentially expressed in anther tissue of a plant and substantially not expressed in non-anther tissue;

(ii) a promoter region capable of directing expression in tapetum and/or pollen tissue;

(iii) a nucleotide sequence substantially as set forth in SEQ ID NO. 5 or having at least 20% similarity to all or part thereof.

10. A genomic DNA isolate according to claim 9 further comprising:

(iv) a nucleotide sequence which is capable of hybridising under low stringency conditions to all or part of a nucleotide sequence substantially complementary to SEQ ID NO. 5.

11. A genomic DNA isolate according to claim 10 comprising a nucleotide sequence as set forth in SEQ ID NO. 5.

12. A genomic DNA isolate according to claim 10 comprising a nucleotide sequence as set forth in SEQ ID NO. 6.

13. An isolated nucleic acid molecule capable of hybridising under low stringency conditions to the genomic DNA isolate according to claim 1 or 4 or 5.

14. An isolated nucleic acid molecule according to claim 13 wherein the nucleic acid molecule is a complementary strand of all or part of SEQ ID NO. 1 or SEQ ID NO. 2.

15. An isolated nucleic acid molecule according to claim 14 in the form of an oligonucleotide.

16. A ribozyme comprising a hybridising region and a catalytic region wherein the hybridising region is capable of hybridising to at least part of a target mRNA sequence transcribed from a genomic DNA isolate according to claim 1 or 4 or 5 wherein the catalytic region is capable of cleaving said target mRNA thereby substantially down regulating expression of said genomic DNA isolate.

17. A ribozyme according to claim 16 wherein in use, said ribozyme renders a target plant substantially male sterile.

18. A genetic construct comprising a promoter region capable of directing expression of a nucleotide sequence when operably linked downstream thereof in tapetum and/or pollen tissue wherein said promoter region comprises a nucleotide sequence substantially as set forth in SEQ ID NO. 3 or having at least 20% similarity to all or part thereof.

19. A genetic construct according to claim 18 wherein the promoter region comprises a nucleotide sequence substantially as set forth in SEQ ID NO. 3.

20. A genetic construct according to claim 18 wherein the promoter region comprises a nucleotide sequence substantially as set forth in SEQ ID NO. 4.

21. A genetic construct according to claim 18 or 19 or 20 comprising at least one unique restriction endonuclease site in a region 3′ of the promoter region to permit insertion of an operably linked nucleotide sequence downstream of said promoter region.

22. A genetic construct according to claim 18 further comprising one or more regulatory regions capable of controlling operation of said promoter region.

23. A genetic construct according to claim 18 wherein the nucleotide sequence downstream of said promoter region is capable of inducing an infertile pollen grain or a pollen grain incapable of maturation.

24. A genetic construct according to claim 18 wherein the nucleotide sequence downstream of said promoter is all or part of Bcp1 or Bgp1 in reverse orientation relative the promoter.

25. A genetic construct comprising:

(i) a promoter region capable of directing expression of a nucleotide sequence when operably linked downstream thereof in tapetum and/or pollen tissue; and

(ii) said promoter being capable of hybridising under low stringency conditions to a complementary strand of SEQ ID NO. 3.

26. A genetic construct according to claim 25 wherein the promoter region comprises a nucleotide sequence substantially as set forth in SEQ ID NO. 3.

27. A genetic construct according to claim 25 wherein the promoter region comprises a nucleotide sequence substantially as set forth in SEQ ID NO. 4.

28. A genetic construct according to claim 25 comprising at least one unique restriction endonuclease site in a region 3' of the promoter to permit insertion of an operably linked nucleotide sequence downstream of said promoter region.

29. A genetic construct according to claim 25 further comprising one or more regulatory regions capable of controlling operation of said promoter region.

30. A genetic construct according to claim 25 wherein the nucleotide sequence downstream of said promoter region is capable of inducing an infertile pollen grain or a pollen grain incapable of maturation.

31. A genetic construct according to claim 25 wherein the nucleotide sequence downstream of said promoter region is all or part of Bcp1 or Bgp1 in reverse orientation relative the promoter.

32. A method for generating male sterile plants, said method comprising transforming a cell or group of cells of said plant with the genetic construct according to claim 18 or 25 wherein said genetic construct directs expression of a nucleotide sequence having a deleterious effect on tapetum and/or pollen tissue, regenerating a transgenic plant from said transformed cells and growing and/or maintaining said transgenic plant under conditions to thereby having a deleterious effect on said tapetum and/or pollen tissue resulting in said plant being substantially male sterile.

33. A method according to claim 32 wherein the nucleotide sequence having a deleterious effect in antisense to all or part of SEQ ID NO. 1 or SEQ ID NO. 2.

34. A method according to claim 32 wherein the nucleotide sequence having a deleterious effect is a ribozyme comprising a hybridising region and a catalytic region wherein the hybridising is capable of hybridising to at least part of a target mRNA sequence transcribed from a genomic DNA isolate according to claim 1 or 4 or 5 wherein the catalytic region is capable of cleaving said target mRNA thereby substantially down regulating expression of said genomic DNA isolate.

35. A transgenic plant comprising a genetic construct capable of substantially down regulating expression of SEQ ID NO. 1 or a nucleotide sequence having at least 20% similarity to all or part thereof such that said transgenic plant is male sterile.

36. A transgenic plant according to claim 35 wherein said plant is a *Brassica* species, *Arabidopsis* species or *Nicotiana* species.

37. A method for generating male sterile plants, said method comprising introducing into a cell or group of cells of said plant a genetic construct comprising all or part of a Bgp1 gene, said Bgp1 gene having a nucleotide sequence substantially similar to an endogenous Bgp1 of the plant and then regenerating a plant from said cells.

38. A method according to claim 37 wherein the introduced Bgp1 gene does not include a promoter region.

39. A method according to claim 37 or 38 wherein the introduced Bgp1 gene comprises a nucleotide sequence as set forth in SEQ ID NO. 1 or 2.

40. An antisense genetic construct comprising SEQ ID NO. 1 or SEQ ID NO. 3 in reverse orientation.

41. An antisense genetic construct comprising a part of SEQ ID NO. 1 or SEQ ID NO. 3 in reverse orientation.

42. A hybrid genetic sequence comprising a ribozyme according to claim 16 or 17 and an anisense genetic construct according to claim 40 or 41.

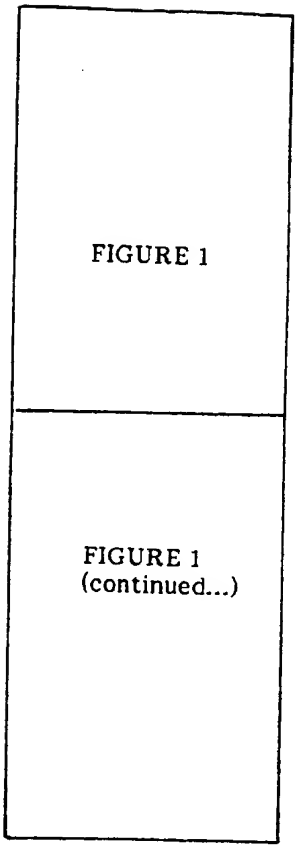
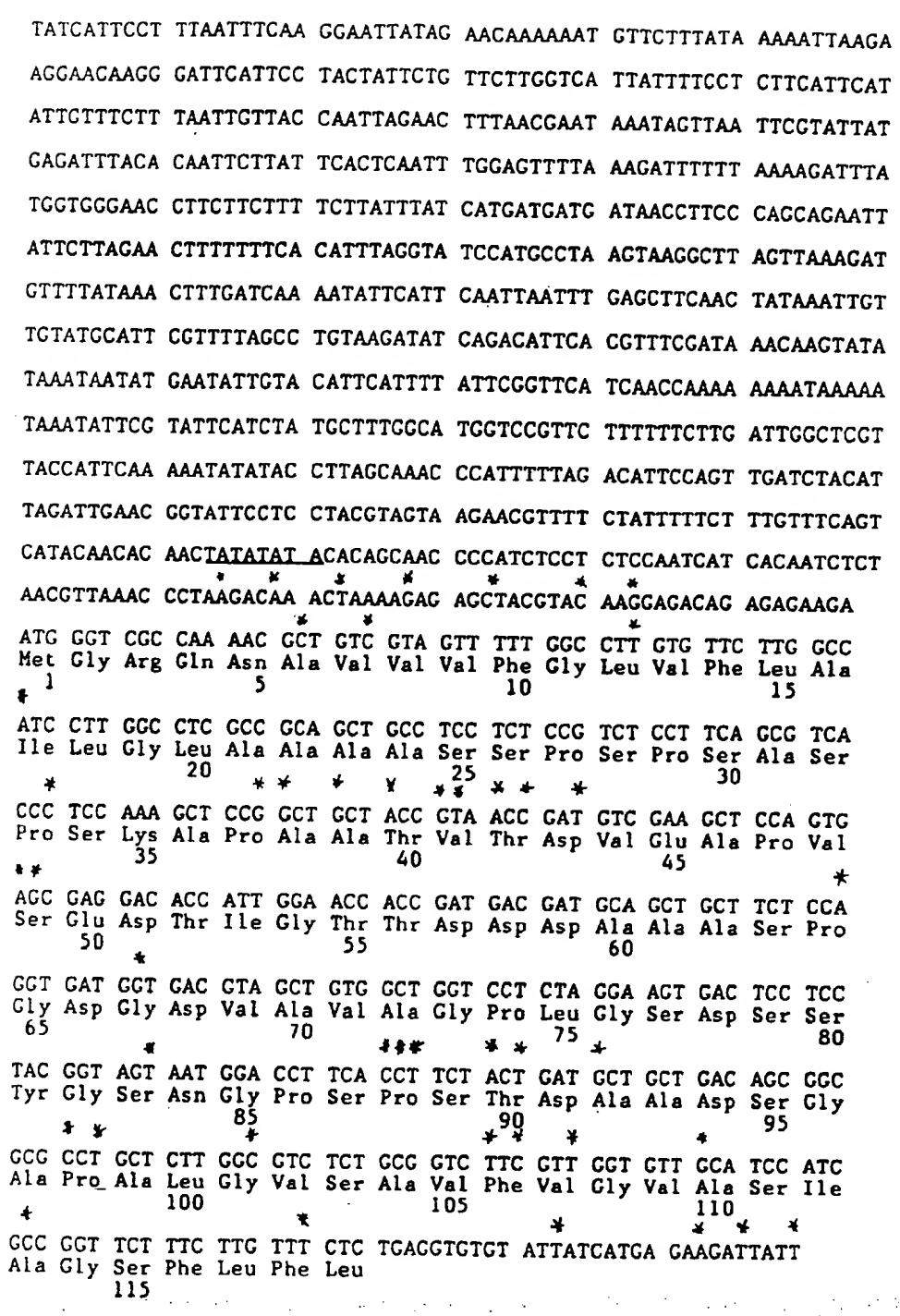


FIGURE 1
FIGURE 1
(continued...)

FIGURE 1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ATTGTTTCTT TAATTGTTAC CAATTAGAAC TTTAACGAAT AAATAGTTAA TTCGTATTAT
GAGATTTACA CAATTCTTAT TCACTCAATT TGGAGTTTTA AAGATTTTTT AAAAGATTTA
TGGTGGGAAC CTTCTTCTTT TCTTATTTAT CATGATGATG ATAACCTTCC CAGCAGAATT
ATTCTTAGAA CTTTTTTTCA CATTTAGGTA TCCATGCCTA AGTAAGGCTT AGTTAAAGAT
GTTTTATAAA CTTTGATCAA AATATTCATT CAATTAATTT GAGCTTCAAC TATAAATTGT
TGTATGCATT CGTTTTAGCC TGTAAGATAT CAGACATTCA CGTTTCGATA AACAAGTATA
TAAATAATAT GAATATTGTA CATTCATTTT ATTCGGTTCA TCAACCAAAA AAAATAAAAA
TAAATATTCG TATTCATCTA TGCTTTGGCA TGGTCCGTTC TTTTTTCTTG ATTGGCTCGT
TACCATTCAA AAATATATAC CTTAGCAAAC CCATTTTTAG ACATTCCAGT TGATCTACAT
TAGATTGAAC GGTATTCCTC CTACGTAGTA AGAACGTTTT CTATTTTTCT TTGTTTCAGT
CATACAACAC AACTATATAT ACACAGCAAC CCCATCTCCT CTCCAATCAT CACAATCTCT
AACGTTAAAC CCTAAGACAA ACTAAAAGAG AGCTACGTAC AAGGAGACAG AGAGAAGA
ATG GGT CGC CAA AAC GCT GTC GTA GTT TTT GGC CTT GTG TTC TTG GCC
Met Gly Arg Gln Asn Ala Val Val Val Phe Gly Leu Val Phe Leu Ala
1 5 10 15
ATC CTT GGC CTC GCC GCA GCT GCC TCC TCT CCG TCT CCT TCA GCG TCA
Ile Leu Gly Leu Ala Ala Ala Ala Ser Ser Pro Ser Pro Ser Ala Ser
20 25 30
CCC TCC AAA GCT CCG GCT GCT ACC GTA ACC GAT GTC GAA GCT CCA GTG
Pro Ser Lys Ala Pro Ala Ala Thr Val Thr Asp Val Glu Ala Pro Val
35 40 45
AGC GAG GAC ACC ATT GGA ACC ACC GAT GAC GAT GCA GCT GCT TCT CCA
Ser Glu Asp Thr Ile Gly Thr Thr Asp Asp Asp Ala Ala Ala Ser Pro
50 55 60
GGT GAT GGT GAC GTA GCT GTG GCT GGT CCT CTA GGA AGT GAC TCC TCC
Gly Asp Gly Asp Val Ala Val Ala Gly Pro Leu Gly Ser Asp Ser Ser
65 70 75 80
TAC GGT AGT AAT GGA CCT TCA CCT TCT ACT GAT GCT GCT GAC AGC GGC
Tyr Gly Ser Asn Gly Pro Ser Pro Ser Thr Asp Ala Ala Asp Ser Gly
85 90 95
GCG CCT GCT CTT GGC GTC TCT GCG GTC TTC GTT GGT GTT GCA TCC ATC
Ala Pro Ala Leu Gly Val Ser Ala Val Phe Val Gly Val Ala Ser Ile
100 105 110
GCC GGT TCT TTC TTG TTT CTC TGAGGTGTGT ATTATCATGA GAAGATTATT
Ala Gly Ser Phe Leu Phe Leu
115

FIGURE 1 (continued...)

CTGACTGAAG ACTATTAATA TGTATGGATG ATTGTGATGG TCGTGTTGTA ATATGTTTCT

CCTTTATTGT GAGAAACGAT GTTTTGCTAA TAAAACTGAA AAAAAAAACG AAAATTTCCT

CTAGCCAAGG ATAAAATGCC GGAATTGCGG ATTAAATAGT ACTATTCAAT CCTTTCATGT

TTTCGAGATA CAAAAATACA TATTAATCAG GTAGAGCCGT AGAAGTCCGT AACCACTGGA

TACAATCTTT TTCGTAGTAA GAAAGAAAGT ACAATCTTAT TCTAAATGCA TGTGTTTGAT

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TACCATCTTT TTAGGTATAG CTTCTTCTTC TATAAA

FIGURE 2

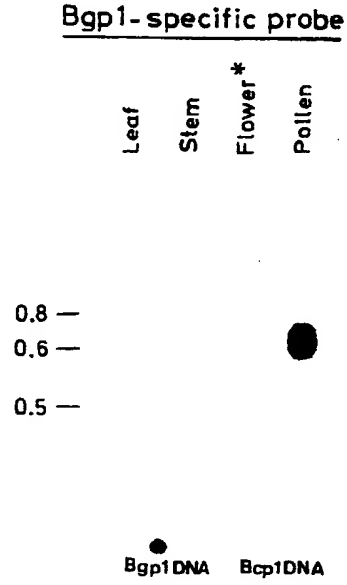

Bgp1-specific probe
Leaf
Stem
Flower*
Pollen
0.8 —
0.6 —
0.5 —
Bgp1DNA
Bcp1DNA

FIGURE 3

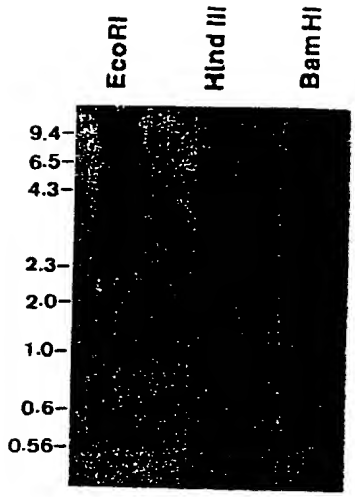


FIGURE 4

FIGURE 5

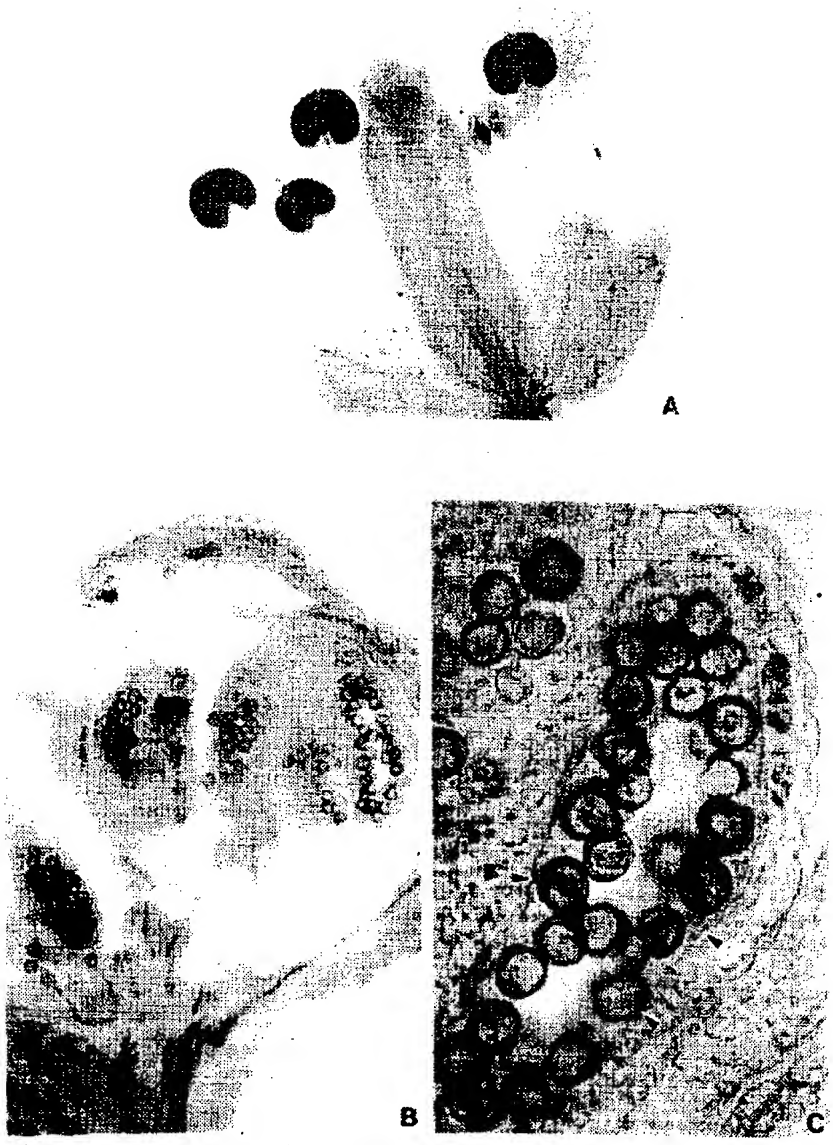


FIGURE 5 (continued...)

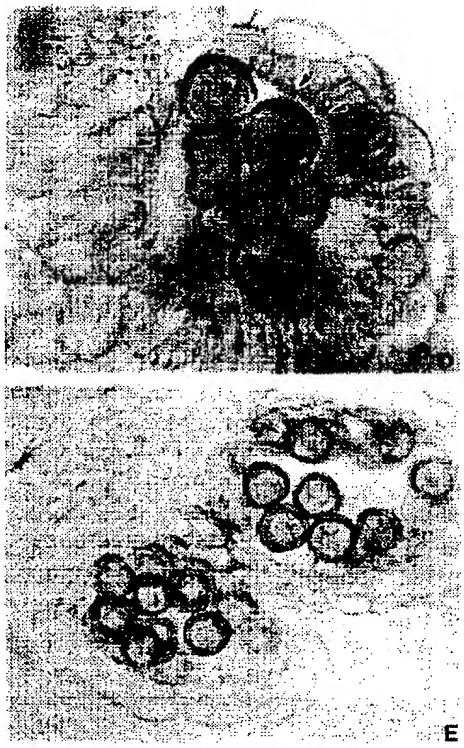
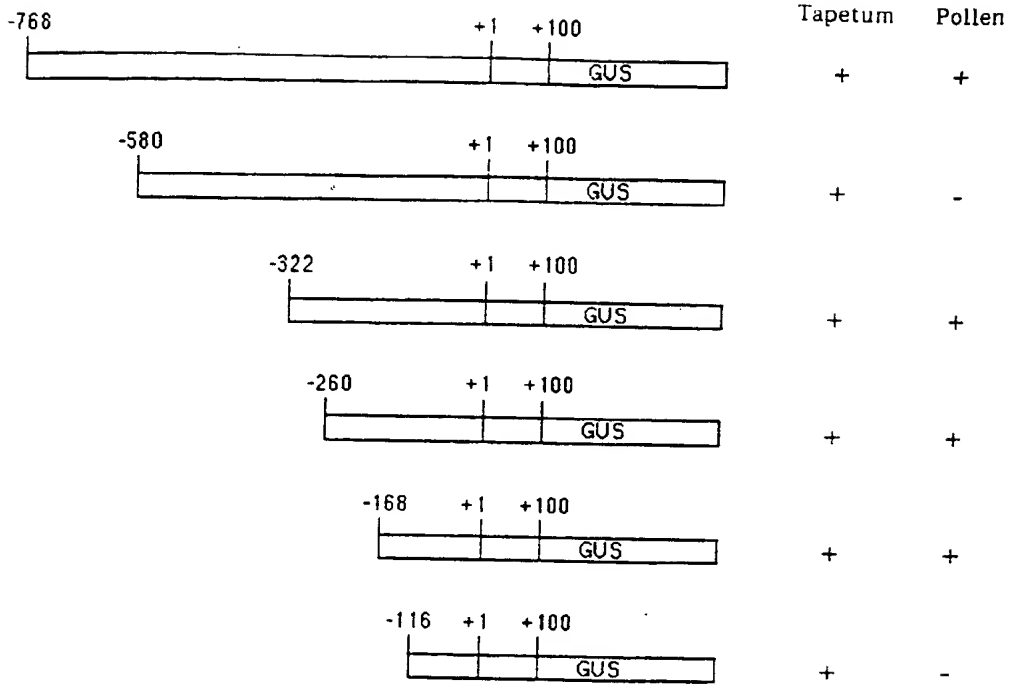


FIGURE 6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GUS
Tapetum
Pollen
+
+
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GUS
+
+
-260
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+
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+
-

FIGURE 7

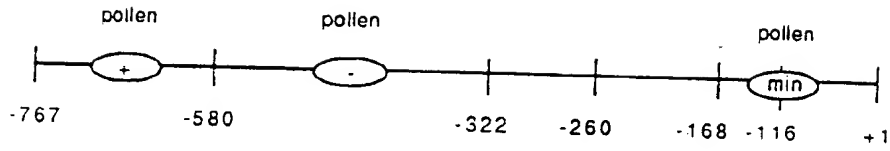
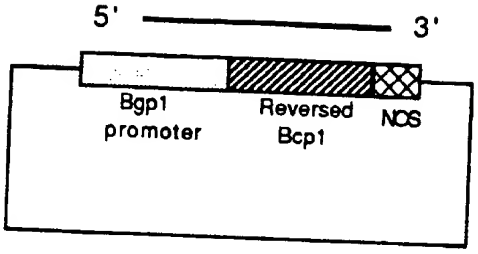


FIGURE 8



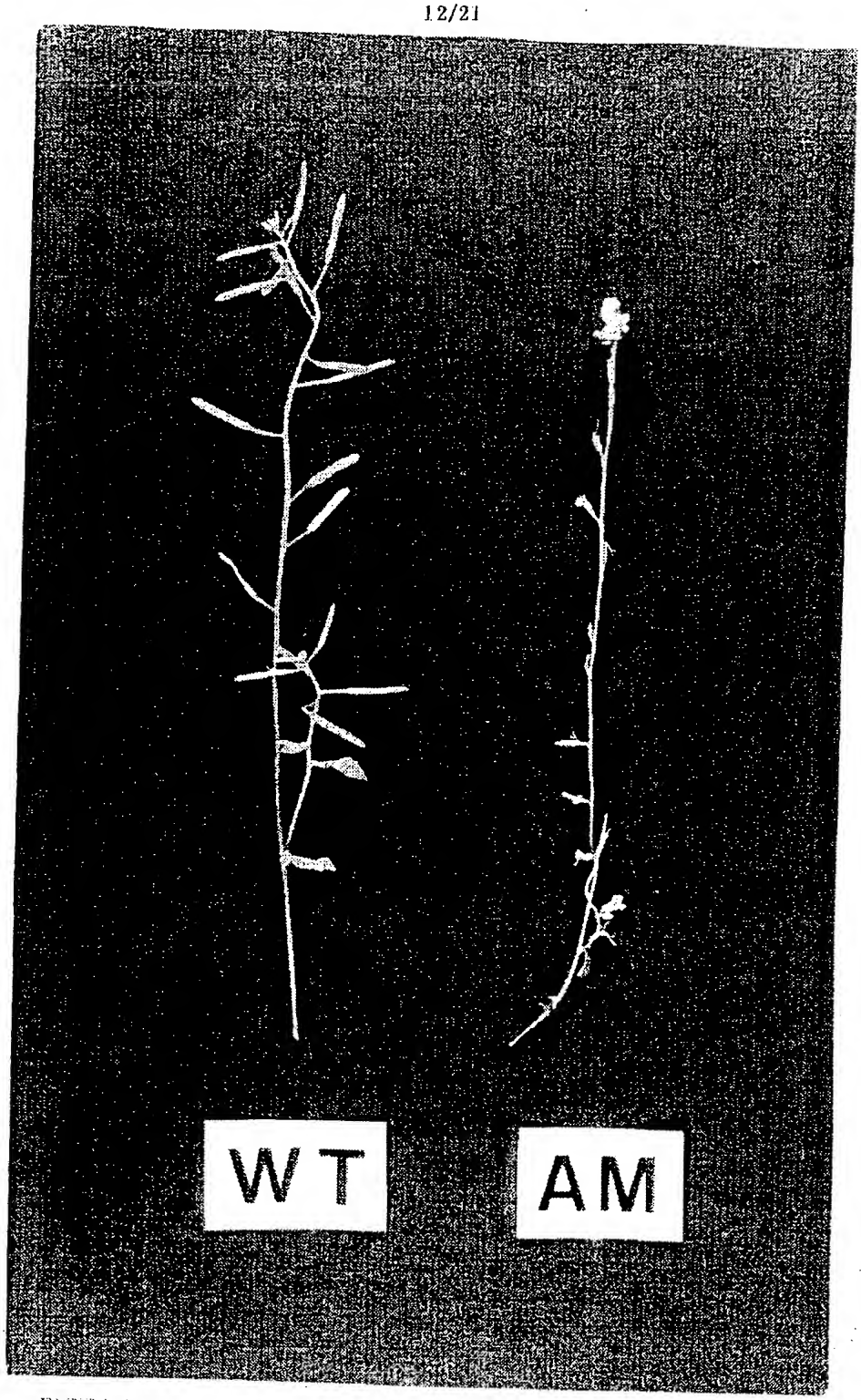
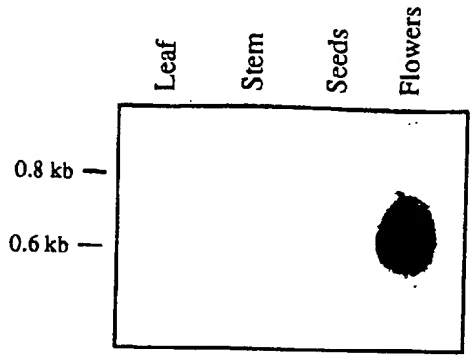


FIGURE 9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Stem
Seeds
Flowers
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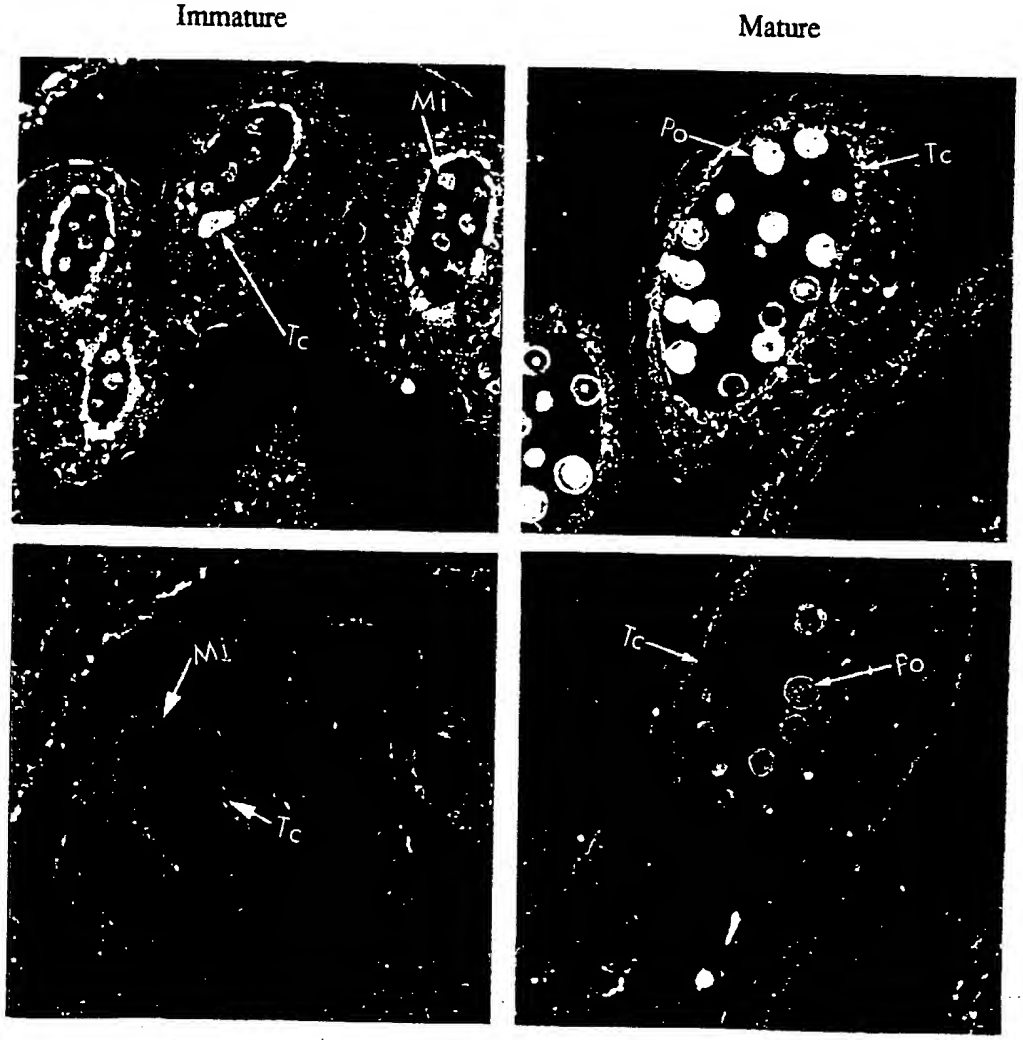
Immature
Mature
Mi
Tc
Po
Tc
Mi
Tc
Tc
Po

FIGURE 10

FIGURE 11B

```
AAAAGCGAGA AGAAGAAGTC TGGAAGATTT GAGAGCTTAA AGTGGTCGAG TGTAAAACCC

TAACTCGCTG TTGATGGCAG AATCGTAAAT CGGAATTGAT TCATGGGCCT AACAAGACGT

TTGGGCTTAT GGGTTTAAAG CCCATCTGAT ATAAGATGAA TAGAATGTTC ATGGCAATAC

TATCATAATT TGGTTCTTTA ATAAGACACT CGTTAATACG ACGACGATTT GAAGTTGAAC

GAATGTTTTC ATATTCATTC GCATGTTCAC CAATCAAAAT CTATATCTGA ACAAGTCCAT

TTTTAGGTAC TCCAGTAGAT TTACATTGGA TTGTAAGGTA ATCCTACATC TTAGTTCACG

TTTTCTATTT TTGGTCTTGT CACTAAACAC AACTATATAT ACATATCAAA CTCATCTTCG

GAAATCATCA CAATCAATAA ACCTCAAACC CTAAAATAAA TTAAACGAGT TCTACGTAAG

AAGGAGAGAG AGAAGA ATG GGT CGC CAA AAC ATT GTC GTC GTG GTT GCC
                 Met Gly Arg Gln Asn Ile Val Val Val Val Ala
                   1               5                  10

CTC GTC TTC ATC CGG ATC ATT GGC CTT GCC GCA GCT GCC TCC TCT CCA
Leu Val Phe Ile Arg Ile Ile Gly Leu Ala Ala Ala Ala Ser Ser Pro
            15                  20                  25

TCT CCT TCA GCG TCT CCC TCC AAA GCT CCA GCT GCC TCC AAA ACC GAT
Ser Pro Ser Ala Ser Pro Ser Lys Ala Pro Ala Ala Ser Lys Thr Asp
        30                  35                  40

CAT GTC GAG GCT CCA GTC ACC GAT GAC CAA ATC GGA ACC ACC GAT GAC
His Val Glu Ala Pro Val Thr Asp Asp Gln Ile Gly Thr Thr Asp Asp
    45                  50                  55

GAT GCA GCT CCT ACT CCT GGT GAC GGT GAC GTT GCA GTG GCT GGT CCT
Asp Ala Ala Pro Thr Pro Gly Asp Gly Asp Val Ala Val Ala Gly Pro
60                  65                  70                  75

CTA GGA AGT GAC TCC TCG TAC GAC AAT GCC GCT ACA GGC TCT GCT GAT
Leu Gly Ser Asp Ser Ser Tyr Asp Asn Ala Ala Thr Gly Ser Ala Asp
                80                  85                  90

TCT GCC AAA AGC GGT GCG GCA GCT CTT GGC GTC TCT GCG GTC GTC GTT
Ser Ala Lys Ser Gly Ala Ala Ala Leu Gly Val Ser Ala Val Val Val
            95                  100                 105

GGT GTT ACA TCA TTG CTG GTT CTT TCT TGT TAC TCA AGT TGG GCA TTG
Gly Val Thr Ser Leu Leu Val Leu Ser Cys Tyr Ser Ser Trp Ala Leu
        110                 115                 120

TTT TAT GAT AAG AAG GTT ATT TTA AAC GAA GAT TAT TAT ATG
Phe Tyr Asp Lys Lys Val Ile Leu Asn Glu Asp Tyr Tyr Met
    125                 130                 135

TAAGGATGAT TGTGATGATC CGTTGACCTG CAGGTCGACC CAGATCCGCC TACCTTTCAC

GAGTTGCGCA GTTTGTCTGC AAGACTCTAT GAGAAGCTGA TAAGAGATAA GTTTGCTCAA

CATCTTCTCG GGCATAAGTC CGGACACCAT GGCATCACAG TATCGAGATG ACAGAGGCAG

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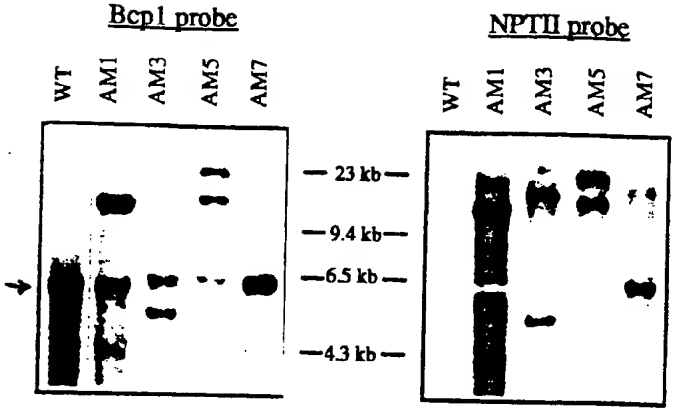
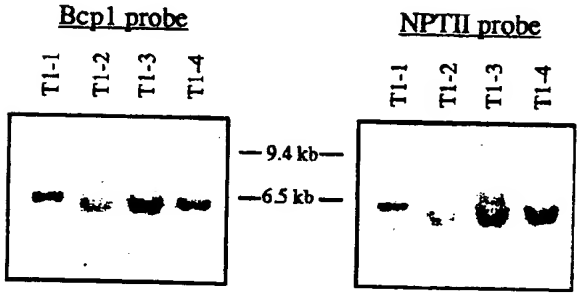
FIGURE 12

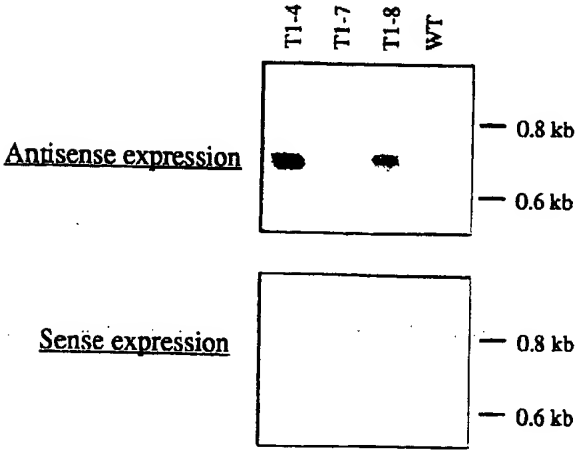
a



b



c



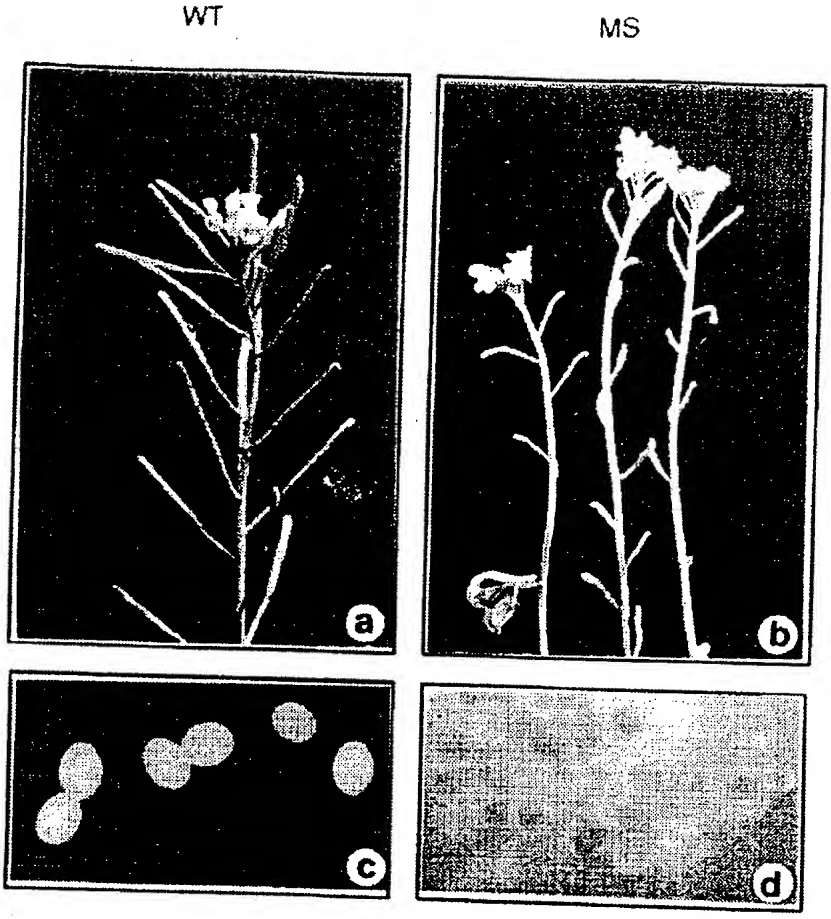


FIGURE 13

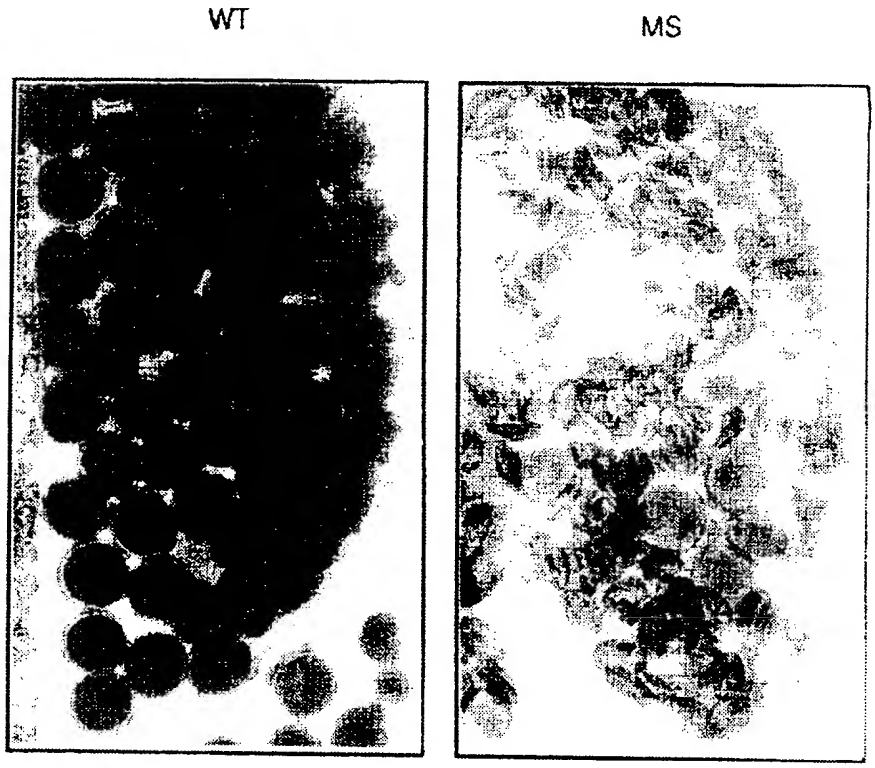


FIGURE 14

FIGURE 15

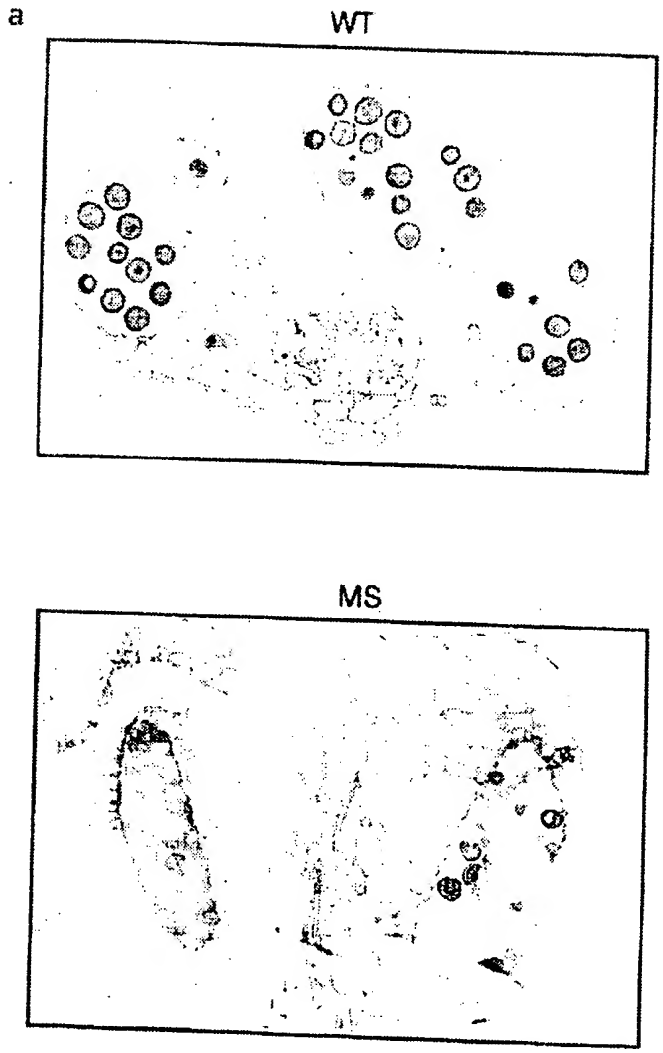


FIGURE 15 (continued...)

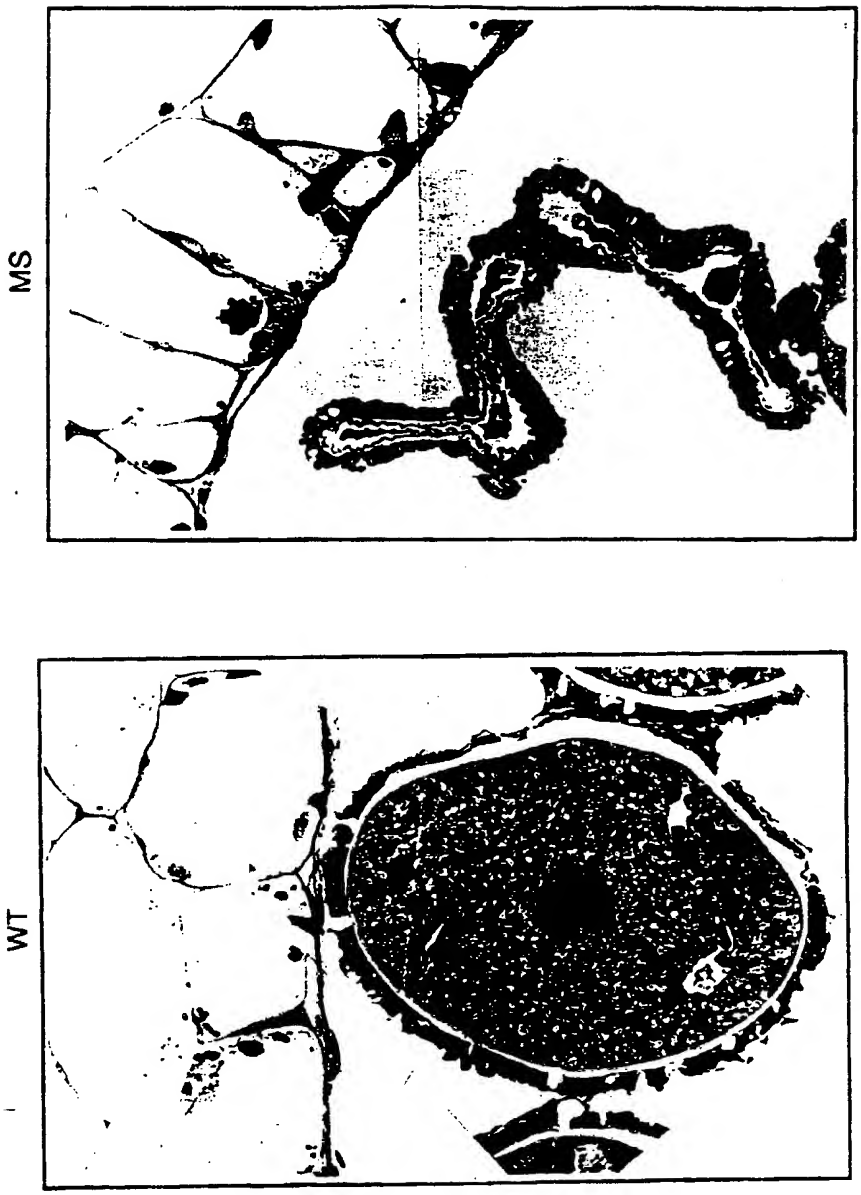
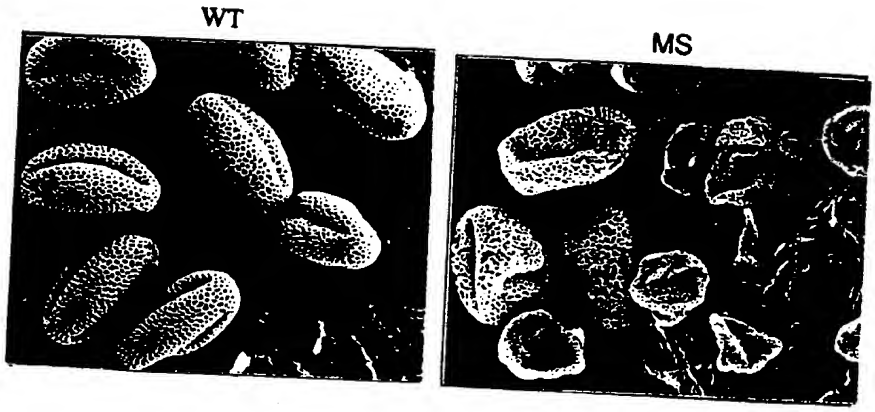


FIGURE 15 (continued...)

C



A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.[5] C12N 15/29, 15/11, A01H 5/50, 1/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC C12N 15/11, 15/29 and keywords below

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC as above

Electronic data base consulted during the international search (name of data base, and where practicable, search terms used)
DERWENT:- WPAT, CASA, BIOT - Brassica, Arabidopsis, nicotiana, tobacco, anther, tapetum, pollen, sterile, Bgpl or DATABASES or Bcpl, C12N/IC, A01H/IC STN - GGT CGCCAA AAC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
PY PA	Nature, Volume 363, 1993, p715-717 Aarts et al. "Transposon tagging of male sterility gene in Arabidopsis"	37-38 25-31, 39-41
PX	Molecular Gen. Genet. Volume 239, No. 1-2, pages 58-65 Huiling et al. "Haploid and diploid expression of a Brassica campestris anther specific gene promotor in Arabidopsis and tobacco"	25-31, 37-41
A	The Plant Cell, Volume 3, pages 1073-1084, October 1991. Theerakulpisut et al. "Isolation and Development Expression of Bcpl, an Anther-Specific cDNA Clone in Brassica campestris"	125-31 and 37-41

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 March 1994 (28.03.94)	12 April 1994 (12.04.94)
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No. 06 2853929	Authorized officer T Richards T RICHARDS Telephone No. (06) 2832445

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate of the relevant passages	Relevant to Claim No.
Y	Journal of Cellular Biochemistry, Molecular Strategies for Crop Improvement, Volume 14E, 1990, page 262 Meriani et al. "Engineered Male Sterility"	37-41
A	Plant: Journal of the Tissue Culture Association, Volume 28 No. 3 part 2 1992 page 51A Leemans et al. "Genetic Engineering for Fertility Control"	37-41
A	Nature, Volume 347 pages 737-41, 1990 Mariani et al. "Induction of Male Sterility in Plants by a Chimeric Ribonuclease Gene"	37-41
Y	WO 90/08828 by Paladin Hybrids Inc, 9 August 1990 (09.08.90)	37-41
Y	WO 92/13957 by Plant Genetics Systems, 20 August 1992 (20.08.92)	37-41
A	EP 0420819 by Max Planck Gesellschaft, 3 April 1991 (03.04.91)	25-31, 37-41

Box I **Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)**

This international search report has not established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claim Nos.: Claims 1-24, 32-36 and 42
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

The claims with the phrase "having at least 20% similarity thereto" and those appended to these are unsearchable because of the infinite number of subsequences of sequence numbers 1 to 6 that are contained within the claims' scope.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically

claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	13957/92	AU	12021/92	CA	2103572	EP	570422
WO	9008828	NZ	227835	AU	50372/90	EP	456706
		JP	4504355	EP	329308	AU	29632/89
EP	420819	AP	9000207	AU	63140/90	CA	2026049
		DE	3931969	HU	905930	HU	57828
		IL	95751	JP	3228684	ZA	9007656

END OF ANNEX